Looking for risk factors for resistance: the correct methodology

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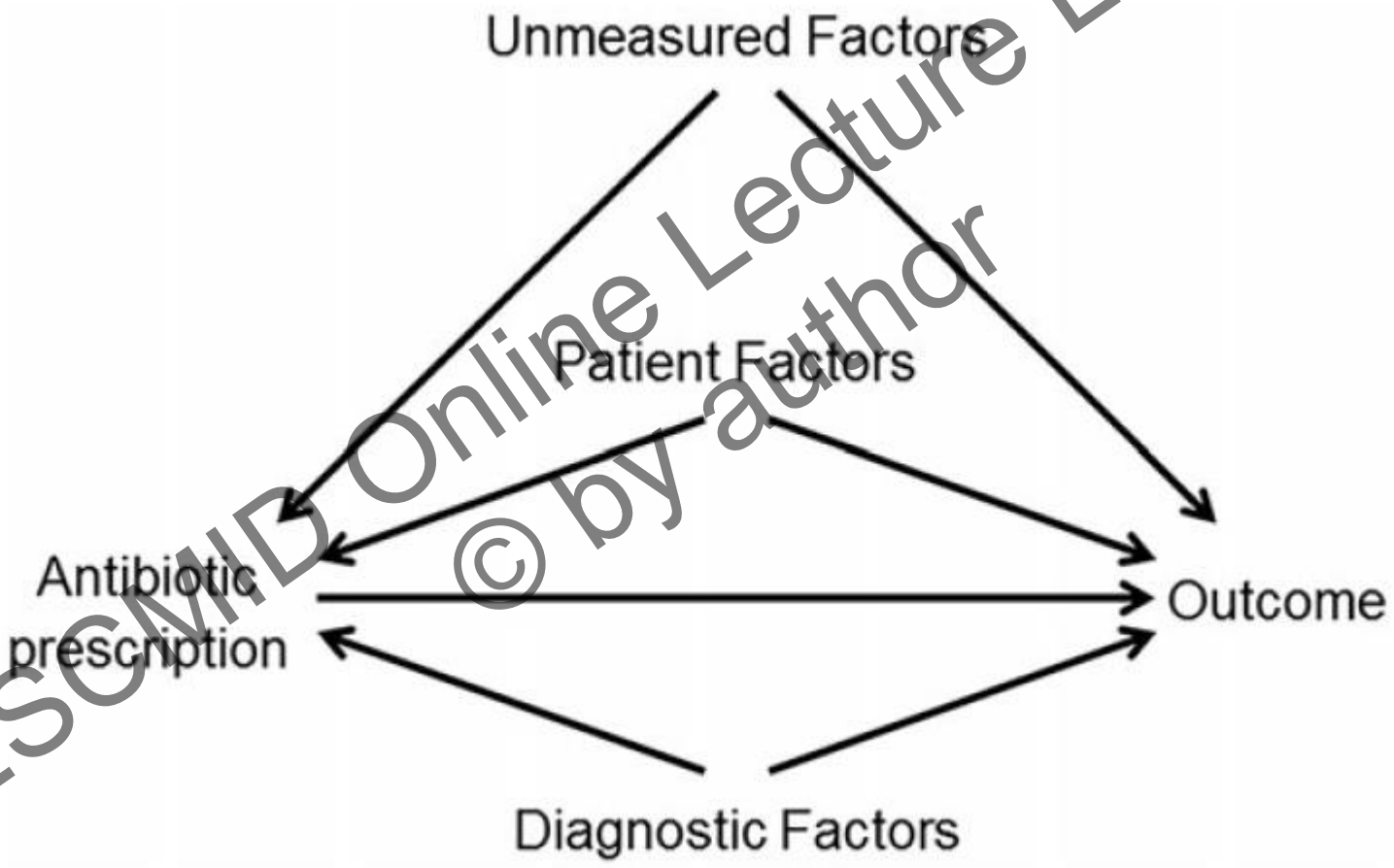
Road map

1. Major limitations of existing evidence
2. Major limitations of most common statistical methods
3. Major limitations of most common study design
4. Hopes

Analysis of retrospective antibiotic usage is one of the most biased issue in infectious diseases



Why?

1. In clinical practice, clinicians prescribe antibiotic treatment based on **the diagnostic** factors related to the disease being treated (eg, culture results, white blood cell count, creatinine), as well as the **prognostic** factors of a particular patient (eg, severity of illness, comorbid conditions).
2. These factors alter physician prescribing for each clinical situation.
3. The specific clinical situation and prescribing patterns are often dependent on known factors as well as unknown or unmeasured factors (ie, factors that are not recorded in the medical record or clinical database).

Why?

4. When the factors that influence the clinician to choose a particular drug are also independently associated with the outcome under study, a failure to control for these factors can lead to a confounding of the true association between the agent prescribed and the outcome.
5. This type of confounding is defined by epidemiologists as confounding by indication.
6. Controlling for severity of illness, type of diagnosis, and patient comorbid conditions is difficult to do, and controlling for other unmeasured confounders is almost impossible.

STROBE Statement

Strengthening the reporting of observational studies in epidemiology

STROBE checklists

Version 4 as published in Oct / Nov 2007:

- STROBE checklist for **cohort, case-control, and cross-sectional studies** (combined)
  download PDF / Word
- Checklist for **cohort studies**
  download PDF / Word
- Checklist for **case-control studies**
  download PDF / Word
- Checklist for **cross-sectional studies**
  download PDF / Word
- Draft STROBE checklist for **conference abstracts**
  download PDF

Antibiotic usage and resistance

Study subgroups	No. of studies	No. of patients	No. of cases	Summary risk ratio (95% CI)	*Q* test of heterogeneity
Study design					
retrospective	20	2344	902	1.9 (1.7–2)	<0.001
prospective	50	20 734	2974	1.8 (1.7–1.8)	<0.001
case–control	31	6145	1806	1.9 (1.7–2)	<0.001
cohort	33	12 496	1942	1.7 (1.6–1.8)	<0.001
prevalence surveys	6	4437	128	1.7 (1.3–2.2)	0.3
Definition of controls					
MSSA-positive	32	5652	2156	1.9 (1.8–2)	<0.001
S. aureus-negative	10	3111	300	1.6 (1.5–1.8)	0.002
mixed population	28	14 315	1420	1.7 (1.6–1.8)	<0.001
Definition of cases					
colonized	27	14 145	1048	1.6 (1.5–1.7)	<0.001
infected	35	6490	2140	1.9 (1.8–2)	<0.001
Sampling frame for inclusion					
nosocomial/HCA-based	30	9544	1490	1.7 (1.6–1.9)	<0.001
community-based	26	8924	1274	1.6 (1.5–1.7)	<0.001
Performed in ICU					
yes	9	4139	452	1.4 (1.3–1.5)	<0.001
no	61	18 939	3424	1.9 (1.8–2)	<0.001
Adjusted for covariates					
yes	10	1643	379	2 (1.8–2.3)	0.001
no	60	21 435	3497	1.8 (1.7–1.8)	<0.001
Adjusted for hospital stay[a]	4	332	109	1.8 (1.4–2.2)	0.07
Performed during MRSA outbreak					
yes	7	899	344	1.9 (1.7–2.1)	<0.001
no	63	22 179	3532	1.8 (1.7–1.9)	<0.001

Tacconelli, JAC (2008) 61, 26–38

Meta-analyses on epidemiological studies on risk factors for antibiotic resistance(I)

Table 1
Examples of meta-analyses on risk factors for infections

First Author, Year of Publication [ref.]	Risk Factor(s)/ Infection (Setting)	Included Studies, n	Study Design	Main Results	Major Limits
Safdar 2008[42]	MRSA colonization/ MRSA infection	10	Clinical trial, case-control, cohort	MRSA colonization increases the risk for MRSA infection	Significant between studies heterogeneity; differences in severity of illness, frequency of sampling to detect colonization, choice of patients population
Tacconelli 2008[13]	Antimicrobial therapy/ MRSA acquisition	76	Case-control, cohort, cross-sectional	Antimicrobial exposure increases the risk for MRSA acquisition	Significant between studies heterogeneity; case definition bias; control selection bias
Baral 2007[27]	MSM/HIV infection (low- and middle-income countries)	83	Convenience samples, cross-sectional	MSM have a greater risk for being infected with HIV	Study design; control selection bias
Chen 2007[28]	Sexual behaviors/HIV infection (Africa)	68	Cross-sectional, case-control, longitudinal	Number of partners, paid sex, HSV-2 infection increase the risk for HIV transmission	Significant heterogeneity of risks; misclassification of exposures; limited choice of risk factors
Weiss 2006[34]	Male circumcision/genital herpes, syphilis, and chancroid	26	Cross-sectional, case-control, cohort	Circumcision decreases the risk for chancroid and syphilis; less association with genital herpes	Significant heterogeneity between studies; misclassification of serologic status and of circumcision status

Tacconelli E, Infect Dis Clin North Am. 2009

Meta-analyses on epidemiological studies on risk factors for antibiotic resistance (II)

Vamvakas 2002[38]	Allogeneic and autologous blood transfusion/ postoperative infections	5	RCTs	No difference in risk for infection	Case definition bias
Thomas 2003[40]	Antimicrobial therapy/ *C difficile* acquisition	48	Cross-sectional, case-control, cohort	Antibiotic exposure increases the risk for *C difficile* acquisition	Control group selection bias; lack of precision in the effect estimates
Bignardi 1998[39]	Risk factors/ *C difficile* acquisition	30	Clinical trial, case-control, cohort	Increasing age, underlying diseases, nonsurgical gastrointestinal procedures, nasogastric tube, antiulcer medications, stay on ICU, duration of hospital stay and antibiotic course, administration of multiple antibiotics increases the risk for *C difficile* acquisition	Control group selection bias; study design; inadequate sample size; inadequate control of confounders; diagnostic bias
Carmeli 1999[43]	Vancomycin use/VRE acquisition	20	Case-control	Vancomycin use increases the risk for VRE acquisition	Significant between studies heterogeneity; control group selection bias

Tacconelli E, Infect Dis Clin North Am. 2009

Restriction

1. The principle of restriction is to try to make the groups being compared more homogeneous with respect to measured factors, thereby removing the possibility of confounding by the measure to which you restricted.
2. Although restriction is a strong tool for factors that are measured, it is unable to control for unmeasured factors.
3. An additional limitation of restriction is that it can dramatically reduce the sample size of the study (multicenter collaborations?).

Matching

1. Matching is a process by which the distributions of important measured factors are actively balanced between the 2 comparison groups
2. There may be a large number of confounding variables that are important and it becomes difficult to match on several variables.
3. Additionally, the problem of unmeasured factors/unmeasured confounding remains.

Stratification

1. Similar to restriction, stratification defines subgroups of patients based on factors that have been measured but without discarding entire strata.
2. Assuming that all confounding factors were measured, stratified estimates can be unbiased. However, assuming that all relevant factors are known and measured is a very strong assumption.
3. Although stratification can be a reasonable option when there are sufficient patients for subgroup analyses, strata with small numbers of subjects can cause challenges, such as reduced power to detect a clinically relevant difference.

Multivariable regression

1. Regression analysis allows for an easier approach to a stratified analysis, particularly when there are multiple confounding factors that you want or need to include in your model to estimate a treatment effect.
2. There are several different types of multivariable regression. Most commonly seen in the literature are regression methods which assume that a parametric distribution fits the data.
3. A basic rule of thumb when creating multivariable regression models is that you should have at least 10 events for each covariate you include in your model.
4. However, they do not alone address the issue of unmeasured confounding.

Propensity score

1. A propensity score is a patient's probability of being exposed to a particular treatment as a function of all of the patient factors that were measured before treatment began that are potentially associated with outcomes.
2. Propensity scores are essentially a summary score of the measured confounders that are created by modeling the probability of exposure as the outcome in a regression model.
3. Propensity scores are a tool to balance the measured factors across exposure (eg, antibiotic) classification

Method	Strengths	Weaknesses
Restriction	Easy to do, easy to understand	Can limit power
Matching	Easy to do, easy to understand	Difficult to match on a large number of variables
Stratification	More powerful than restriction and matching	Difficult to deal with strata with small numbers. Can introduce residual confounding if subjects with very different values of a factor are grouped into a strata (eg, quartiles)
Multivariable regression	More powerful than restriction and matching	Model building can be complicated Can introduce residual confounding if subjects with very different values of a factor are grouped into a strata (eg, quartiles)
Propensity scores	Can be used to assess amount of unmeasured confounding that exists	Not designed specifically to deal with unmeasured confounding
Instrumental variables	One of the newer, more powerful methods	Difficulty in finding the optimal instrumental variable

Type of epidemiological studies

1. Clinical observations (case series)
2. Cross-sectional studies
3. Case-control studies
4. Cohort studies

Case-control study

1. Investigates prior exposure of individuals with a particular health condition and those without
2. Great potential for bias
3. Rare condition (< 5% of population)
4. Major disadvantages:
   Select cases and controls after both the outcome and the assumption of risk have occurred

Case-control definition

1. If strict criteria are not employed to define precisely what constitutes a case, the case group may be diluted with non-cases resulting in spurious associations

2. Including cases of a different disease with a different etiology in the case group can increase the probability of a type Ⅱ error (finding no association when one really exists)

3. It can require multiple level of evidence (clinical, diagnostic, and microbiological)

4. It can be multi-classified (i.e. suspect, probable, and definite)

Case-control definition

1. Cases and control may be selected retrospectively or prospectively but always during the same respective time periods

2. One advantage to selecting cases and controls retrospectively is that the investigator can go back as far as needed to get a sufficient number of subjects to maintain a desired level of power in the study

3. Disadvantages: exposure data on intake forms may be incomplete or unreliable; change in diagnostic methods or disease classifications

Control selection

1. To achieve comparability between cases and controls, the controls should come from the same population that generated the cases.
2. Risks: selection bias or confounding
3. Most frequent matching criteria: race, sex, age group, comorbid, from the same hospital, and during the same time period
4. Depending on the specific hypothesis being tested, there may be other factors that need to be controlled to minimize potential confounding or selection bias

Hospital-based case-control studies

1. Studies that select cases from clinical facilities are commonly referred to as hospital-based case-control studies
2. These cases are usually not representative of all possible cases in the general population because of differences in disease severity or socioeconomic factors related to hospitalization
3. This can affect external validity, but it will not affect internal validity if the controls are similar to the cases
4. Restriction, when properly applied, does not invalidate case-control studies as long as it is applied equally to the controls

Population-based case-control studies

1. Cases and controls are based on a total or representative sample of a defined population
2. Less common than hospital-based studies because of the time and expense involved in identifying eligible subjects and obtaining the required data
3. Cases obtained from these studies, however, should be representative of those in the target population
4. One way of identifying incident cases for population-based studies is to utilize a population-based disease registry

Population-based case-control studies

1. Controls from population-based case-control studies are usually selected randomly from the source populations. These controls should represent the actual exposure rate of subjects without the study disease in the source population
2. Potential selection bias due to low rates of participation and possible measurement bias due to poor recall
3. Neighbours, family members, friends, of coworkers from the source population. These types of controls may improve participation and reduce recall bias, but they may also introduce a negative bias in the study results because the controls may be too similar to the cases with regard to exposure status

Definition of case

TABLE 3. Differences in Independent Risk Factors Between Patients Harboring Ciprofloxacin-Resistant *Pseudomonas aeruginosa* (CRPA) and Those Harboring Multidrug-Resistant *P. aeruginosa* (MDR-PA)

Patient group	Adjusted OR (95% CI)	*P*
CRPA group		
Nonambulatory status	5.6 (1.4-23)	.02
Quinolones[a]	5.0 (1.2-21)	.03
MDR-PA group		
Charlson score of >2	3.3 (1.8-6.0)	<.001
Quinolones[a]	2.8 (1.2-5.0)	.001
Cephalosporins[a,b]	3.5 (1.7-7.1)	<.001
Carbapenems[a]	3.8 (1.2-12.1)	.02
Gentamicin[a]	2.3 (1.04-5.1)	.04

D'Agata & Tacconelli, ICHE, 2006

Control selection

Table 1. Assessment of 3 methodological principles of case-control studies among 37 studies that analyzed risk factors for antibiotic resistance.

Methodological recommendation	Adherence with recommendation, no. (%)			
	Yes	No	Uncertain	Not applicable
Control group selected from study base	13 (35%)	24 (65%)	0	0
Time at risk assessed	11 (30%)	22 (60%)	0	4 (11%)
Comorbid illnesses assessed	27 (73%)	8 (22%)	0	2 (5%)

Harris, CID 2002, 34,

Table 2. Multivariable analyses of risk factors for the isolation of vancomycin-resistant enterococci.

Control group, risk factor	OR (95% CI)
2a[a]	
Use of vancomycin	4.38 (3.24–5.93)
Use of aminoglycosides	1.99 (1.45–2.74)
Use of piperacillin-tazobactam	2.10 (1.56–2.84)
Use of first-generation cephalosporin	0.52 (0.35–0.77)
Use of second-generation cephalosporin	0.41 (0.22–0.78)
Use of quinolones	1.66 (1.26–2.19)
Time at risk, days	1.02 (1.01–1.03)
Surgery	0.69 (0.50–0.96)
2b[b]	
Use of vancomycin	2.86 (2.19–3.73)
Use of aminoglycosides	1.92 (1.43–2.57)
Use of piperacillin-tazobactam	1.99 (1.51–2.62)
Use of ampicillin-sulbactam	2.44 (1.55–3.86)
Use of third-generation cephalosporins	1.81 (1.33–2.47)
Use of quinolones	1.40 (1.09–1.80)
Time at risk, days	1.03 (1.02–1.04)
Surgery	1.47 (1.06–2.06)
Transfer from a different hospital	1.60 (1.20–2.13)
Intensive care unit stay	1.97 (1.53–2.54)

[a] Patients with vancomycin-susceptible enterococci.
[b] Randomly selected patients.

Control selection

Harris, CID 2002, 34, 1558

ESBL+E. coli and Klebsiella spp Antibiotic classification

Table 4
Multivariate analyses using 'class' and 'spectrum' categorisations

Variable	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	*P*-value
Final multivariate model: antibiotic class categorisation			
Use of third-generation cephalosporin	16.0 (2.00–127.92)	1.35 (0.10–17.73)	0.82
Use of vancomycin	7.15 (2.36–21.63)	8.74 (2.09–36.46)	0.003
Duration of hospitalisation prior to ESBL-EK infection	1.03 (1.01–1.05)	1.03 (1.01–1.05)	0.03
Renal insufficiency	11.56 (2.59–51.67)	3.94 (1.00–15.60)	0.05
Final multivariate model: antibiotic spectrum categorisation			
Use of antimicrobial agent active against *Pseudomonas aeruginosa*	14.82 (3.40–64.66)	3.70 (0.49–27.79)	0.20
Use of antibiotic active against Gram-negative organisms	27.90 (3.72–209.24)	10.27 (1.21–86.47)	0.03
Duration of hospitalisation prior to ESBL-EK infection	1.03 (1.01–1.05)	1.01 (0.98–1.03)	0.31
Central venous catheter	11.56 (2.59–51.67)	5.48 (0.63–48.08)	0.12

OR, odds ratio; 95% CI, 95% confidence interval; ESBL-EK, extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella* spp.

MacAdam, International JAA 28 (2006) 325–332

Control selection

Table 1. Multivariable analyses of risk factors for the isolation of imipenem-resistant *Pseudomonas aeruginosa*.

Control group, risk factor	OR (95% CI)
1a[a]	
Use of imipenem	27.12 (13.91–52.90)
Use of aminoglycosides	2.38 (1.40–4.05)
Use of quinolones	3.25 (1.92–5.49)
Surgery	0.42 (0.21–0.85)
1b[b]	
Use of imipenem	6.34 (3.66–11.00)
Use of aminoglycosides	3.28 (1.98–5.42)
Time at risk, days	1.03 (1.01–1.04)
Intensive care unit stay	3.85 (2.16–6.86)

[a] Patients with imipenem-susceptible *P. aeruginosa*.
[b] Randomly selected patients.

Harris, CID 2002, 34, 1558

Control selection

Table 3. Multivariable analyses of risk factors for the isolation of ampicillin-sulbactam–resistant *Escherichia coli*.

Control group, risk factor	OR (95% CI)
3a[a]	
Use of ampicillin-sulbactam	2.71 (1.64–4.46)
Use of quinolones	2.72 (1.16–6.37)
3b[b]	
Age	1.01 (1.00–1.03)
Use of ampicillin	2.69 (1.08–6.69)
Use of ampicillin-sulbactam	1.68 (1.02–2.77)
Use of first-generation cephalosporins	0.31 (0.18–0.53)
Male sex	0.33 (0.23–0.48)
Hepatic disease	1.89 (1.08–3.32)
Intensive care unit stay	2.42 (1.62–3.63)
Surgery	2.07 (1.36–3.17)
Transfer from a different hospital	1.41 (0.96–2.07)

[a] Patients with ampicillin-sulbactam–susceptible *E. coli*.
[b] Randomly selected patients.

Harris, CID 2002, 34, 1558

Cohort study

1. Exposure precedes the health outcome
2. Expensive, time consuming and most ligistically difficult of all studies
3. Useful for relatively common diseases

Advantages

1. They clearly demonstrate an appropriate temporal sequence between exposure and outcome
2. They permit the direct calculation of incidence rates in both the exposed and unexposed groups
3. Easy to calculate risk or rate ratios as needed
4. Permit multiple outcomes to be assessed
5. They can be used to study exposures that are relatively uncommon

Disadvantages

1. Potential large sample size requirements
2. Long follow-up periods
3. Need to reassess exposure on a frequent basis
4. Outcomes must be determined as they develop
5. Potential losses to follow-up
6. Potential exposure misclassification
7. Possible outcome misclassification

Retrospective cohort studies

1. A sufficient number of the individuals in the cohort must have been exposed to high enough levels of the factor that an adequate number of outcomes are likely to have occurred
2. Must be a sufficiently accurate source of existing data on exposure levels
3. It must be possible to identify an appropriate comparison group
4. The comparison group is usually an external comparison group
5. It is important to have available sources from which to identify potentially confounding factors in the study and comparison groups

Antibiotic Use for Acute Respiratory Infections

Table 4. Severe Adverse Events Per 100,000 Visits by Ant

Groupe			Value
All antibi			.54
Specific a			
β-Lacta			.37
Macrolides	2.40	−3.26 to 8.07	.40
Flouroquinolones	1.06	−17.02 to 19.14	.91

- ✓ Inaccuracy of the system
- ✓ Information about real use of ATB missing
- ✓ ATB prescription is not randomised

Retrospective cohort patients with ARI visits (UK primary care database).
Exposure: antibiotic prescribed with the visit.
Primary outcomes were hospitalization within 15 days for (1) severe adverse drug events or (2) CAP

Meropol, Ann Fam med 2013

Cross-sectional studies

1. Compares groups in terms of their current health and exposure status and assess their similarities
2. Easy to conduct
3. Disadvantage: a cause cannot be inferred because only current health and exposure are being studied

Background. Upper respiratory tract infections (URTIs) are common in children. The cause of URTIs is usually viral, but parents' attitudes often contribute to inappropriate prescription of antibiotics, promoting antibiotic resistance. The objective of this study was to identify possible risk factors associated with antibiotic misuse in Greece, a country with high levels of antibiotic use and antibiotic resistance. *Methods*. A knowledge-attitude-practice (KAP) questionnaire was developed and distributed to Greek parents caring for children who were 5-6 years old, between January and July of the same school year. *Results*. The sample of the study contained 5312 parents from all geographic areas of Greece. The risk factors of being a father, having low education, having immigrant status, being a single parent, having low income, having <2 or >3 children, living in the islands, and being without experience in recurrent URTIs were significantly associated to inadequate knowledge, inappropriate attitudes, and wrong practices. *Conclusions*. This study has identified the main groups of parents that should be targeted in future intervention programs.

- ✓ Heterogeneity in case distribution
- ✓ Parents' self-report about their knowledge
- ✓ Language and medical terms used

Panagaku ISRN 2013

Conclusions
Most frequent mistakes

1. Erroneous selection of the control group, matching criteria
2. Lack or erroneous definitions
3. Lack of definition of exposure
4. Lack of adjustment
5. Subgroup analysis missing
6. Different length of follow up

Conclusions
Must to control!

- Combination therapy and sequential therapy
- Comorbidity
- Context where data are gathered (outbreak or endemic)
- Patients population
- Infection versus colonisation
- Length of hospitalization
- Site of acquisition