National Food Institute DTU
Technical University of Denmark

NEWSLETTER

to the
National Reference Laboratories for Antimicrobial Resistance

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Community Reference Laboratory Antimicrobial Resistance

In This Issue

With this newsletter the EU Community Reference Laboratory for Antimicrobial Resistance present for you three very relevant subjects for the work with antimicrobial resistance. The subjects focus on the basics of antimicrobial susceptibility testing with the aim of having the same terms of reference within this subject area. This is especially essential due to the increased globalisation and the focus on harmonisation that we experience.

Content

Methods for testing *Salmonella* and *Campylobacter*

Frank M. Aarestrup, Patrick F. McDermott

In general there are two different methods for testing the susceptibility of a bacterial isolate to antimicrobial agents, dilution and diffusion methods. Several variations of both methods have been used worldwide for susceptibility testing of numerous bacterial species, including *Salmonella* and *Campylobacter*. This includes a number of diffusion (disk, tablets, Etest) and dilution (macro- and micro-broth, and agar dilution) methods.

Salmonella belong to the Enterobacteriaceae, are fast growing and do not require special nutritional supplements in the growth medium. *Campylobacter* are slow growing and require microaerobic conditions and supplemented media for growth. Thus, large variations in different media used and incubations conditions, including different atmospheres and temperatures and time of incubation have been employed. Since the MIC or inhibition zone can vary greatly depending on the method used it is important to either only compare studies using comparable methods or ensure the use of optimum break points for the different methods used.

The ideal susceptibility testing method should give reproducible results between laboratories and over time. In addition, it would be desirable if it is easy to perform and cheap. Correctly performed susceptibility

testing requires continuous quality control of the entire procedure, including quality of the media, incubation conditions and temperature. An essential part of the quality control is the use of quality control strains with known and defined susceptibilities. Laboratories should adhere to a standard, well-defined method that includes the necessary quality control information.

A number of different organizations publish standards for antimicrobial susceptibility testing. The most widely used are probably those published by CLSI (www.clsi.org), EUCAST (www.eucast.org) and ISO (www.iso.org).

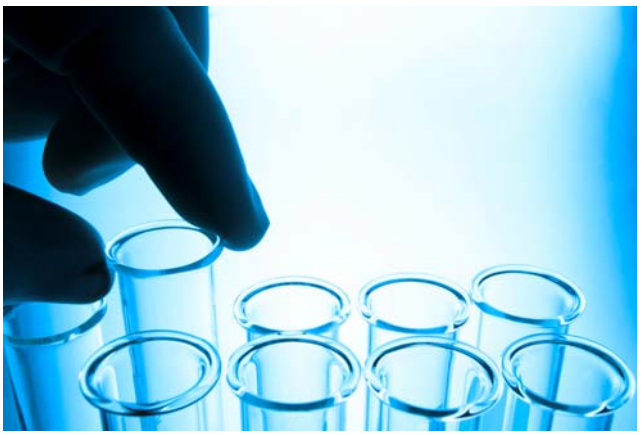
Comparable standards for how to perform both dilution and diffusion susceptibility testing for Enterobacteriaceae are available from all three organizations. The clinical break points suggested might differ, but the standards should give the same result.

An agar dilution method for susceptibility testing of *Campylobacter* has only recently been developed and recognised by CLSI. The guidelines give detailed information on media and incubation conditions, including temperature and time. Currently the agar and micro broth dilution methods are the only internationally recognised methods for susceptibility testing of *Campylobacter*. A number of studies have compared different methods for susceptibility testing of *Campylobacter*. These studies have in general showed a good agreement between test methods when the break points for categorising isolates into susceptible and resistant were based on experience within the individual laboratories. However, it is currently not possible to correlate the exact MIC values obtained from e.g. Etest and micro broth dilutions. Thus, further standardisation of diffusion methods are needed before these can be recommended for susceptibility testing of *Campylobacter*.

Suggested further reading

McDermott PF, Bodeis-Jones SM, Fritsche TR, Jones RN, Walker RD. 2006. Broth microdilution susceptibility testing of *Campylobacter jejuni* and the determination of quality control ranges for fourteen antimicrobial agents. J Clin Microbiol. 2005 Dec;43(12):6136-8.

J.L. Watts and C.J. Lindeman. 2006. Antimicrobial Susceptibility Testing. In: F.M. Aarestrup, Editor, Antimicrobial Resistance in Bacteria of Animal Origin, ASM Press, Washington, DC, USA (2006) ISBN 1-55581-306-2, pp. 29–35.



1st International Meeting on Antimicrobial Resistance in Zoonotic Bacteria and Food Borne Pathogens

The 1st International Meeting on Antimicrobial Resistance in Zoonotic Bacteria and Food Borne Pathogens that will be held at Scandinavia Radisson hotel in Copenhagen from June 15-18, 2008 are now developing the scientific program. An exclusive group of well-estimated scientists, including members of the industry from around the world is putting together a well-balanced program. This program is expected to be formalized by the end of October and a second announcement will follow soon after this.

If you wish to receive the second announcement, please contact Senor Scientist Lars B. Jensen (lje@food.dtu.dk).

Please see ASM's website for more information (link)

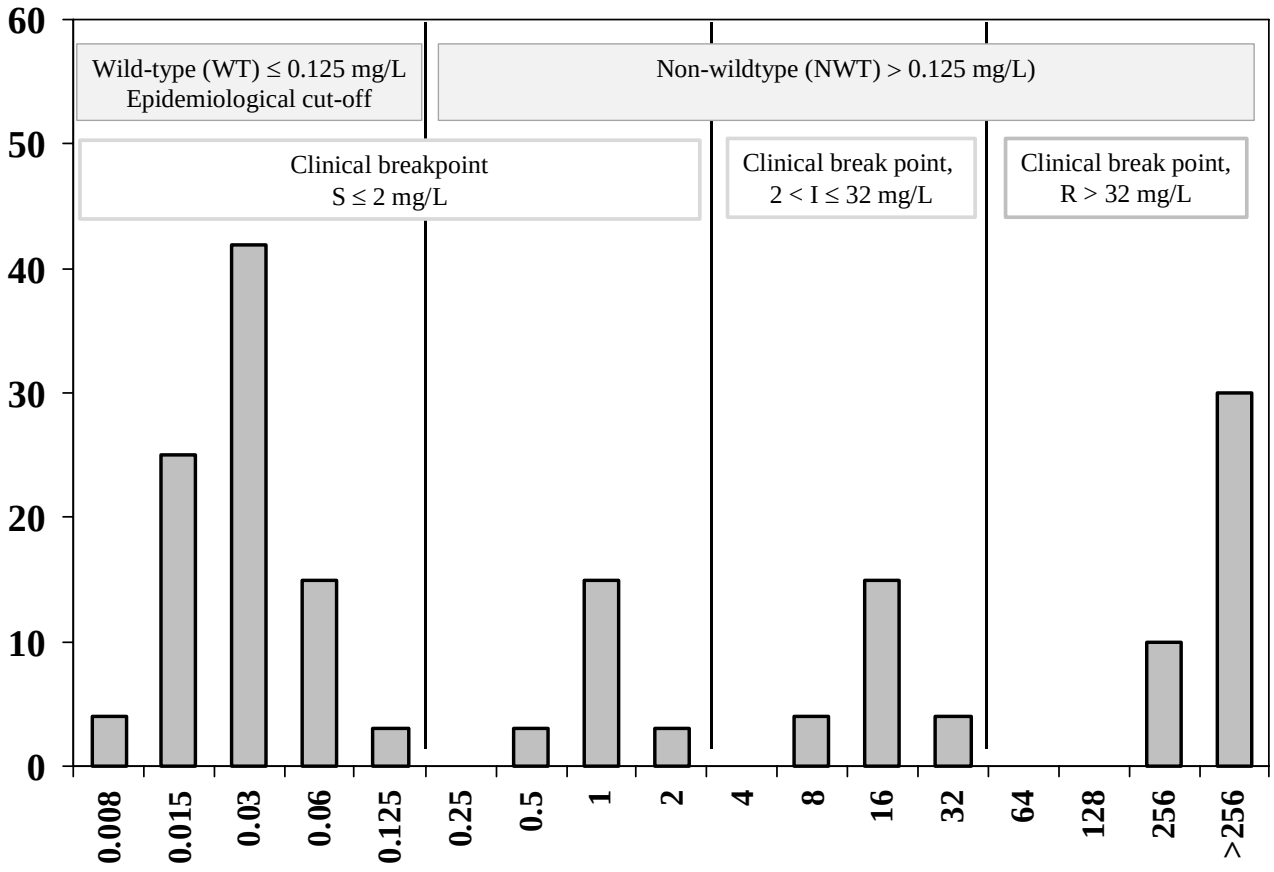
Antimicrobial susceptibility testing

Clinical break points and epidemiological cut-off values

Frank M. Aarestrup, Patrick F. McDermott, Gunnar Kahlmeter

Determination of the antimicrobial susceptibility of a bacterial isolate is important to ensure appropriate therapy of infections in animals and humans and to produce monitoring data on the occurrence of acquired resistance among bacteria in different reservoirs. Semi-quantitative methods for determining the minimum inhibitory concentration (MIC) of an antimicrobial agent for a given bacterial pathogen is the gold standard for susceptibility testing. The use of MIC values to correctly categorize bacterial isolates as susceptible or resistant - also called interpretive criteria - is essential for guidance of correct clinical therapy and for comparing the results from different monitoring programs.

However, interpretive criteria differ between laboratories and countries and with the purpose of the MIC-determination. Thus, MIC break points appropriate for predicting clinical efficacy might differ from those used for monitoring purposes. An isolate might, through mutations or horizontal gene transfer, develop reduced susceptibility to a given drug but still have a sufficiently low MIC to allow successful therapy. Thus, for monitoring purposes the isolate might be considered resistant, but clinically susceptible. It is therefore important to differentiate between MIC interpretative criteria used for clinical purposes (clinical break points) from those used for monitoring (epidemiological cut-off values). A constructed example is given in the figure below.



WT = Wild type organisms, i.e micro-organisms without phenotypically detectable antimicrobial resistance to the drug in question
S = Susceptible; I = Intermediate; R = Resistant

Clinical break points

The development of clinical break points requires microbiological MIC data, generated using standardized in vitro testing methods, pharmacokinetic and pharmacodynamic information and most importantly outcome data from clinical efficacy trails. These three types of data taken together usually are sufficient to establish interpretative criteria for individuals likely to respond when treated with that agent at the approved dosages (susceptible organisms), and those likely to fail therapy when treated with the approved dosage (resistant organisms). The "intermediate" category is used as a buffer zone for to account for day-to-day variability in in vitro antimicrobial susceptibility testing, to provide flexibility for sites of infection where the agent is concentrated, or for agents where increased dosage ranges are defined.

Epidemiological cut-off values

Epidemiological cut-off values focus on separating isolates in the normal wild type population from isolates with some type of acquired mechanisms that reduces the normal susceptibility of these isolates. Thus, the epidemiological cut-off does not take into considerations any data on dosages or clinical efficacy, but is aimed at optimizing the phenotypic detection of isolates with acquired resistance. When working with epidemiological cut-off values there is not an intermediate category; isolates are recorded as wild type or non-wild-type. Epidemiological cut-off values are only used for the monitoring of antimicrobial resistance, and since wild type MIC-distributions of bacteria of human and animal origin coincide completely, the same epidemiological cut-off can be used for monitoring resistance in humans and animals and in different animals.

Sources of clinical break points and epidemiological cut-off values

Several national and international committees determine clinical break points. The most widely used are those provided by the Clinical Laboratory Standards Institute (CLSI, www.clsi.org), which publishes methods for susceptibility testing and tables with clinical break points, both MIC-tables and zone diameter tables as approved by the Food and Drug Administration (FDA) in the USA. In Europe the European Committee for Antimicrobial Susceptibility Testing (EUCAST, www.eucast.org) provides epidemiological cut-off values, clinical breakpoints and the huge database of MIC-distributions needed to determine epidemiological cut-off values. The data is freely available on the EUCAST website but currently only available for MIC-values. There are major differences in the clinical break point for cefotaxime, whereas the clinical break point for ciprofloxacin is the same. In contrast, epidemiological cut-off values are, with few exceptions, lower than the clinical break point.

There is a confusing difference in the use of operators. Thus, CLSI defines the resistant break point as greater than or equal to (≥) an MIC value, whereas EUCAST defines the resistance break point as greater than (>) an MIC value. This difference is based on traditional interpretations of the test outcome. An MIC is defined as the lowest drug concentration that visibly inhibits bacterial growth. For example because MICs are determined by serial two-fold drug dilutions, the true MIC of an isolate which can grow at 1 mg/L, but not 2 mg/L (recorded MIC 2 mg/L), lies somewhere between 1mg/L and 1.99 mg/L. It is not possible to determine the actual MIC when only concentrations of 1 and 2 are tested.

Some differences in resistance breakpoints are exemplified for *Salmonella* in the table below.

Antimicrobial agent	CLSI	EUCAST	
	Clinical break point (R≥; mg/L)	Clinical break point (R>; mg/L)	Epidemiological cut-off (NWT>; mg/L)****
Ampicillin	32	8***	4
Cefotaxime	64	2	0.5
Ciprofloxacin	4	2	0.064
Gentamicin	16*	4	2
Sulphamethoxazole	512	ND**	256***
Tetracycline	16	4***	8

*: Not recommended for treatment of *Salmonella* infections by CLSI; **: Not defined; ***: Preliminary; ****: NWT = Non-Wildtype

Monitoring, ring trials and interpretative criteria

Since the criteria for categorising bacteria as resistant or susceptible; often differ between breakpoint committees, may differ for purposes of treating infections in humans and animals or for different animals, and may depend on dosage, type of infection and may change over time, epidemiological cut-off values offer a common, stable "breakpoint" for the sensitive measuring of phenotypically detectable antimicrobial resistance. For monitoring purposes and in future external quality controls (ring trials) the Community Reference Laboratory for Antimicrobial Resistance and WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens will recommend and use epidemiological cut-off values as provided by EUCAST, as the reference standard for all organisms and antimicrobials.

It should be made clear that the epidemiological cut-off values recommended by EUCAST and those by other organisations, such as CLSI are not always the same. Obviously, these efforts should be coordinated in a continuous harmonisation within this area.

References

Cornaglia G, Hryniewicz W, Jarlier V, Kahlmeter G, Mittermayer H, Stratchounski L, Baquero F; ESCMID Study Group for Antimicrobial Resistance Surveillance. 2004. European recommendations for antimicrobial resistance surveillance. Clin Microbiol Infect 10: 349-383.

Kahlmeter G, Brown DF, Goldstein FW, MacGowan AP, Mouton JW, Osterlund A, Rodloff A, Steinbakk M, Urbaskova P, Vatopoulos A. 2003. European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria. J Antimicrob Chemother 52: 145-148.

Turnidge J, Kahlmeter G, Kronvall G. 2006. Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. Clin Microbiol Infect 12: 418-425.

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Tasks of an EU national reference laboratory

The tasks of an EU national reference laboratory (NRL) are described in 'Regulation (EC) No 882/2004 article 33' and includes the following responsibilities:

The NRL is expected to

- Coordinate the activities for official laboratories in the member state, and to be available for reference purposes with regard to antimicrobial resistance.
- Ensure the quality of the susceptibility testing performed in all official food and veterinary laboratories in the member state. Especially for data submitted to the Community (eg. EFSA) where it is essential that data are obtained in a standardized way
- To organize comparative tests for the official laboratories. As means of the responsibility of the NRL to ensure the quality of susceptibility testing in the member state it is expected that the NRL conducts proficiency tests
- To ensure dissemination of information supplied by the CRL to the official laboratories in the member state
- To provide scientific support to the member state's competent authority

Also, it is expected that the NRL collaborates with the CRL, including

- Taking part in the annually organised proficiency tests for susceptibility testing of *Campylobacter*, *Salmonella*, enterococci, staphylococci and *E.coli* arranged by the CRL
- One annual participation in the workshop arranged by the CRL for the NRL's. At the workshop the participants take part in discussions on matters of relevance for harmonisation of susceptibility testing in the member countries and discuss plans to improve the results of the NRL´s in the proficiency tests
- If necessary, taking part in individual meetings or training courses

Please note that the tasks of the NRL's are not limited to the above mentioned, please see 'Regulation (EC) No 882/2004 article 33' for a full description.