**Rapid disk diffusion antibiotic susceptibility testing allows the discrimination of clinically important resistance phenotypes after 6h to 8h of incubation**

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**Abstract**

**Background:**

**Methods:**

**Results and Conclusions:**

**Introduction**

Detection of important antibiotic resistance mechanisms such as ESBL, AmpC beta-lactamases, carbapenemases, MRSA, or inducible *erm*MLS is critical to ensure a successful clinical outcome. [1-5](#_ENREF_1" \o "Oteo, 2010 #382) [ESBL: Pfaller, Oteo, Pitout, AmpC: Siu, alles andere: EUCAST Resistance mechs] Rapid implementation of an effective, targeted antibiotic treatment significantly improves clinical outcome and reduces mortality. [6-8](#_ENREF_6" \o "Buehler, 2016 #2631) (Ferrer, Kumar, Buehler) Automated microdilution antibiotic susceptibility testing (AST) provides results within 6h to 12h, but carries several disadvantages such as non-flexible drug panels, few drug concentrations tested, the need of a separate purity check, or hardly detected synergism/antagonism phenomena, and a comparably low sensitivity/specificity for the detection of important resistance mechanisms like ESBLs, carbapenemases, or inducible *erm*-mediated MLS. [9-11](#_ENREF_9" \o "Fisher, 2009 #425) Molecular detection of resistance determinants is rapid in principle, but hampered by the vast number of resistance mechanisms to cover. Molecular AST tests by nature are focused on specific genetic elements, making maintenance of an accurate coverage and, hence, detection of the most relevant resistance genes a laborious task considering the different epidemiologies worldwide. [12](#_ENREF_12" \o "EUCAST, 2016 #2385) In addition, the sole presence of genes does not necessarily correlate with expression and phenotypic resistance. Disk diffusion is still an affordable, accurate, reliable, and highly standardised AST method with the advantages of low consumable costs and flexible drug panels. Procedures to reliably detect important resistance mechanisms and their associated phenotypes using disk diffusion AST have been established, but EUCAST and CLSI recommend an incubation time of 16h to 18h for most species drug combinations. [5](#_ENREF_5" \o "Testing, 2013 #1817), [13-15](#_ENREF_13" \o "Maurer, 2015 #1913) (EUCAST Res. Mech; Maurer, Polsfuss) We recently demonstrated that early reading of disk diffusion is principally possible by using automated systems, but major and very major interpretation errors resulted, if current EUCAST CBPs were applied pointing to mandatory adaptions for rapid disk diffusion AST. [16](#_ENREF_16" \o "1,  #2630) (ZIT MS1).

This study aimed at i) analysing the potential of rapid disk diffusion AST, i.e. early zone diameter reading at 6h to 12h, to discriminate important resistance phenotypes from wild-type populations, and ii) describing species-drug combinations, for which CBP changes will be mandatory for early reading due to changes of wild-type zone diameters over time. Diameter changes of the wild-type will result in shifts of corresponding epidemiological cut offs (ECOFFs), which are a critical parameter in CBP setting.

**Methods**

**Quality control strains**

To ensure methodological precision and calibration to EUCAST CBPs, *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 EUCAST quality control strains were tested daily from individual fresh sub-cultures and individually prepared McFarland 0.5 standards. Interpretation and was done according to EUCAST QC tables version 6.1 (EUCAST QC 6.1) [17](#_ENREF_17" \o "Testing., 2016 #2391) (data not shown). Quality control ranges and targets were fulfilled during this study.

**Clinical isolates.**

Study isolates were selected covering a broad range of inhibition zone diameters for each species/drug combination tested. In particular, critical isolates close to the CBPs were included.All non-duplicate clinical strains included in this study were isolated over a 3-year-period from 2013 until 2016 in the clinical microbiology laboratory of the Institute of Medical Microbiology, University of Zurich. Isolates of the same species were considered duplicate(s) if they i) originated from the same patient, and ii) showed one major AND two minor differences in AST interpretation at maximum. The following numbers of clinical isolates were tested: *Escherichia coli* (N=475), *Klebsiella pneumoniae* (N=376), *Enterobacter cloacae* (N=301), *Staphylococcus aureus* (N=394), and *Staphylococcus epidermidis* (N=294).

**Definition of phenotypes.**

Resistance phenotypes and the wild-type populations were defined according to criteria given inTable 1. Assignment of phenotypes was done prior to this study based on independent disk diameter measurements generated by using the EUCAST recommended method on Müller-Hinton II agar (Beckton-Dickinson, Franklin Lakes, NJ, USA) and with antibiotic discs from i2a (i2a, Montpellier, France) and applying EUCAST CBPs (EUCAST 2016). Inhibition zone diameters were recorded automatically using the Sirscan/Sirweb system (i2a, Montpellier, France). Phenotypic screening and confirmation for the detection of AmpC cephalosporinases, ESBLs, and carbapenemases by combination disk testing was performed as previously described (IMM ZITATE). Wild-type definitions for the Enterobacteriaceae and beta-lactams were applied as previously described (ECOFF method Valsesia et al).

**Automated susceptibility testing.**

Susceptibility testing was performed as described previously according to EUCAST guidelines version 6.0, which are essentially the same as that of CLSI 2016 [18](#_ENREF_18" \o "CLSI, 2016 #1897), [19](#_ENREF_19" \o "EUCAST, 2016 #2102) (+ZITAT MS1). In brief, bacterial suspensions were manually adjusted to 0.5 McFarland and processed within 15 minutes. Mueller-Hinton II agar plates (Oxoid Limited, Basingstoke, United Kingdom) were processed in the fully automated WASPTM (Copan Italia, S.p.A., Brescia, Italy), i.e. plates were each inoculated with 60 µl of the bacterial suspension and automatically streaked. Antibiotic discs of a single production lot (Oxoid Limited, Basingstoke, United Kingdom) were placed using a standard distributor, which was handled by the WASPTM robot immediately after plate streaking. Subsequently, plates were automatically transported to and incubated in a WASPLabTM incubator (Copan) at 37°C +/- 2°C in ambient air. Images were taken after 6h, 8h, 12h and 18h of incubation under continuous temperature conditions. Diameter measurements were automatically done by the WASPLabTM reading software (Copan) and were, if necessary, adjusted on-screen by an experienced technician.

**Software.**

Calculations were done using Microsoft Excel 2010 software (Microsoft Corporation, Redmond, WA, USA) and R, version 3.2.3.

**Results**

**Discrimination of major resistance phenotypes from the wild-type.**

In the following, a phenotype is called “well separable” from the wild-type if separation with sensitivity and specificity of at least 0.95 is possible, i.e. if the bars in Supplemental Figure 1 do not overlap. “Fully separated” means separation with sensitivity and specificity 1 is possible, i.e. the whiskers in Supplemental Figure 1 do not overlap, and “not separable” covers remaining cases.

**Beta-lactams**

**Enterobacteriaceae.** Ampicillin susceptibility generally discriminated beta-lactamase producers from the wild-type independently from the reading time (accounts for *E. coli* only as *K. pneumoniae* and *E. cloacae* harbour chromosomal SHV and AmpC enzymes, respectively, Figure 1, Supplemental Figure S1). ESBL producers were separated from the wild-type at 6h to 8h by cefpodoxime or ceftriaxone diameters independent of the bacterial species. Cefoxitin was discriminative for the presence of acquired AmpC beta-lactamases in *E. coli* and *K. pneumoniae*, and meropenem susceptibility at early reading times was useful to separate carbapenemase class A, B, and D enzymes, i.e. KPC, NDM, or OXA-48 producers in the three *Enterobacteriaceae* species analysed. Temocillin was found to be a good marker drug to separate OXA-48 enzymes.

**Staphylococci.** Benzylpenicillin diameters did not separate *bla*Z penicillinase producing *S. aureus* from the wild-type at 6h to 8h while discrimination after 18h was possible (Figure 2, Supplemental Figure S1). MRSA were separated at 6h to 8h from methicillin-susceptible *S. aureus* by cefoxitin, whereas for *S. epidermidis* MRSE and non-MRSE populations showed a diameter overlap at all reading times.

**Fluoroquinolones**

**Enterobacteriaceae.** Nalidixic acid did not separate the low-level fluoroquinolone resistant non-wild-type populations from the wild-types at 6h to 8h for K. pneumoniae and E. cloacae, whereas for E. coli this discrimination was possible at early reading times. High-level fluoroquinolone resistant non-wild-type populations were separated from the corresponding wild-type populations at 6h to 8h for all fluoroquinolones tested and all Enterobacteriaceae species (Figure 1, Supplemental Figure S1).

**Staphylococci.** Norfloxacin was discriminative for low-level and high-level fluoroquinolone resistant non-wild-type populations from the wild-types of S. aureus and S. epidermidis at 6h to 8h (Figure 2, Supplemental Figure S1).

**Aminoglycosides**

**Enterobacteriaceae.** The most prevalent aminoglycoside non-wild-type populations in this study were the AAC(2’)/(AAC(3’)-II/-IV phenotype (conferring resistance to gentamicin), the ANT(4’)/AAC(6’)-Ib phenotype (conferring resistance to gentamicin and tobramycin) and the phenotype group containing multiple resistance mechanisms (Figure 1, Supplemental Figure S1). These non-wild-type and wild-type populations could readily be discriminated at 6h to 8h in all *Enterobacteriaceae* species tested.

**Staphylococci.** The most prevalent aminoglycoside non-wild-type populations in this study were the APH(3’) phenotype (conferring resistance to kanamycin), the ANT(4’)/APH(3’) phenotype (conferring resistance to kanamycin and tobramycin) and the phenotype group containing multiple resistance mechanisms (Figure 2, Supplemental Figure S1). These non-wild-type and wild-type populations could readily be discriminated at 6h to 8h in *S. aureus* and *S. epidermidis*.

**Tetracyclines**

**Enterobacteriaceae.** Clear separation of the non-wild-type from the wild-type by tigecycline was present at all reading times in *E. coli* but not in K. pneumoniae and *E. cloacae* (Figure 1, Supplemental Figure S1).

**Staphylococci.** The non-tetB-efflux phenotype was discriminated from the wild-type by tetracycline at 6h to 8h in *S. aureus* but not in *S. epidermidis*, whereas the “tetB efflux and/or ribosomal protection” phenotype could be discriminated in both staphylococcal species at all reading times (Figure 2, Supplemental Figure S1). Clear separation of the “tetB efflux and/or ribosomal protection” phenotype by minocycline was only detected for *S. epidermidis* after 18h. Tigecycline non-wild-type staphylococcal isolates were not available in this study.

**MLS drugs.**

**Staphylococci.** Erythromycin was discriminative for the cMLS and iMLS phenotype for all reading times and both staphylococcal species analyzed (Figure 2, Supplemental Figure S1). For clindamycin the cMLS non-wild-type of S. aureus was separated from the wild-type at all reading times, whereas in S. epidermidis separation was detected after 18h only. The iMLS phenotype could not be discriminated by diameter values at all reading times but induction phenomena (D-shape test) could already be detected at early reading times.

**Miscellaneous drugs.**

**Enterobacteriaceae.** Trimethoprim-sulfamethoxazole non-wild-type populations could be discriminated from the wild-type at all reading times for E. cloacae and E. coli, while for K. pneumoniae discrimination was possible after 12h to 18h only (Figure 1, Supplemental Figure S1).

**Staphylococci.** Trimethoprim-sulfamethoxazole and rifampicin non-wild-type populations could be discriminated from the wild-type at all reading times for S. epidermidis, while for S. aureus discrimination was possible after 18h only. Fusidic acid non-wild-type populations could be discriminated from the wild-type at all reading times for S. epidermidis, while for S. aureus discrimination was possible after 8h to 18h only (Figure 2, Supplemental Figure S1).

**Change of zone diameters over time**

For the majority of species-drug combinations the zone diameters of the wild-type population increased over time (69 out of 84 combinations, see Table 2). For ten species-drug combinations the zone diameters of the wild-type population remained stable over time, whereas for five species-drug combinations the wild-type zone decreased. Of note, increasing zone diameters were detected for all species-drug combinations and staphylococci analysed. The relative diameter changes over time were mostly consistent between the five species tested with the exceptions of nalidixic acid, for which *E. coli* wild-type zone diameters increased and *K. pneumoniae* and *E. cloacae* diameters decreased, and for trimethoprim-sulfamethoxazole displaying decreasing wild-type zone diameters for Enterobacteriaceae, but increasing zone diameters for staphylococci.

**Discussion**

Rapid disk diffusion AST allowed the discrimination of important resistance phenotypes that are frequently encountered in our clinical laboratory from the corresponding wild-type populations. Rapid detection of resistance mechanisms was possible for important drug classes used in sepsis patients, e.g. the beta-lactam antibiotics and ESBL, carbapenemases, MRSA, high-level fluoroquinolone resistance, constitutive macrolide resistance (MLS), or for major aminoglycoside-modifying enzymes in both *Enterobacteriaceae* and staphylococci. [20](#_ENREF_20" \o "Perner, 2016 #2632) (Penner) For other drug classes the main resistance phenotypes, i.e. the non-wild-type populations could not reliably be separated from the wild-type population after 6h to 8h of incubation, e.g. for tetracyclines, sulfa drugs, rifampicin, or fusidic acid (Figures 1 and 2, Supplemental Figur S1). The latter drugs, however, do not depict first line antibiotics that are empirically administered in the emergency room if sepsis is suspected. Therefore, rapid availability of AST results seems less important as compared to beta-lactams, fluoroquinolones, macrolides, or aminoglycosides.

Basically the same expert rules that are used after 16h to 24h of incubation for the detection of important resistance mechanisms do similarly account for early AST reading after 6h to 8h, e.g. i) cefpodoxime and/or ceftriaxone were the best predictors for the presence of ESBL; ii) cefoxitin was the single best predictor for the presence of an acquired AmpC type beta-lactamase; iii) meropenem was the most sensitive marker for the presence of any type of carbapenemase; iv) for *S. epidermidis* cefoxitin CBPs may need an investigation zone to ensure optimal sensitivity for MRSE; v) norfloxacin can be used to extrapolate fluoroquinolone susceptibility in staphylococci; vi) inducible *erm*MLS-based clindamycin resistance needs additional parameters like the D-shape test to be detected. [5](#_ENREF_5" \o "Testing, 2013 #1817), [13-15](#_ENREF_13" \o "Maurer, 2015 #1913), [18](#_ENREF_18" \o "CLSI, 2016 #1897), [19](#_ENREF_19" \o "EUCAST, 2016 #2102), [21](#_ENREF_21" \o "Jost, 2016 #2656) (IMM Publikationen, EUCAST Resistance mechanisms und EUCAST Expert rules, EUCAST CBP, CLSI CBPs). This study found only few exceptions in which standard 18h AST expert rules could not be extrapolated to early reading times: E.g. benzylpenicillin-based discrimination of *bla*Z harbouring *S. aureus* that were largely overlapping the *S. aureus* beta-lactam wild-type population at 6h to 8h, or the use of *Enterobacteriaceae* nalidixic acid as the indicator drug for low-level fluoroquinolone resistance (Figure 1). [22](#_ENREF_22" \o "EUCAST, 2010 #530) (EUCAST Version 1.1)

This study was not intended to issue general rapid AST guidelines. We used our local epidemiology as a paradigmatic example to investigate the challenges of rapid disk diffusion AST. Aggregated datasets from different laboratories and geographies will be needed for early reading resembling the process of CBP setting that is conducted for standard incubation times by committees such as EUCAST (EUCAST SOP). [23](#_ENREF_23" \o "EUCAST, 2013 #1090) Considering our data we suggest to analyse rapid disk diffusion data in a systematic way: First, it should be checked, if non-wild-type strains can safely be discriminated from the wild-type population at early reading times (Figures 1 and 2, Supplemental Figur S1). If such discrimination is unreliable as e.g. demonstrated in this study for benzylpenicillin and *bla*Z-harbouring *S. aureus*, rapid disk diffusion of the according species-drug combination should be discouraged. Second, given that non-wild-type strains can safely be discriminated from the wild-type populations, it should be analysed, if wild-type zone diameters and the corresponding ECOFFs shift over time (Table 2). If wild-type diameters and ECOFFs are stable, current EUCAST CBPs may readily be applied as depicted in this study by the examples of ampicillin and *E. coli*, or for piperacillin/tazobactam in *K. pneumoniae* and *E. cloacae*. If ECOFFs change, an according CBP change will be necessary for early reading, as shown for the vast majority of species-drug combinations tested (69/84, i.e. 82% of combinations with increasing diameters, 5/84, i.e. 6% of combinations with decreasing diameters).

In summary this study demonstrates that i) the most important non-wild-type populations can be separated from the wild-type at early reading times; ii) the same expert rules do similarly account for early reading as for standard incubation; iii) for the majority of species-drug combinations CBP adaptations at early reading times will be necessary.

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**Transparency declarations**

The Institute of Medical Microbiology, University of Zurich and Copan Italia, S.p.A. cooperate in the development of automated AST. ECB is a consultant of Copan Italia. MH received a travel grant from Copan Italia.

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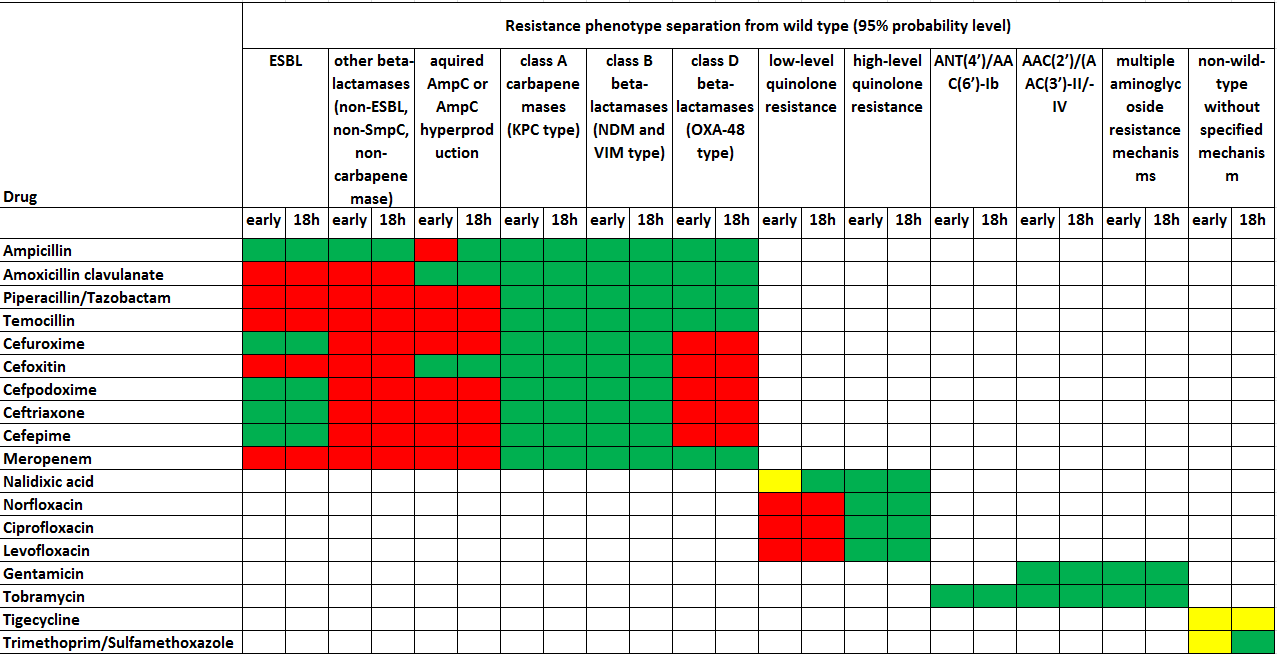
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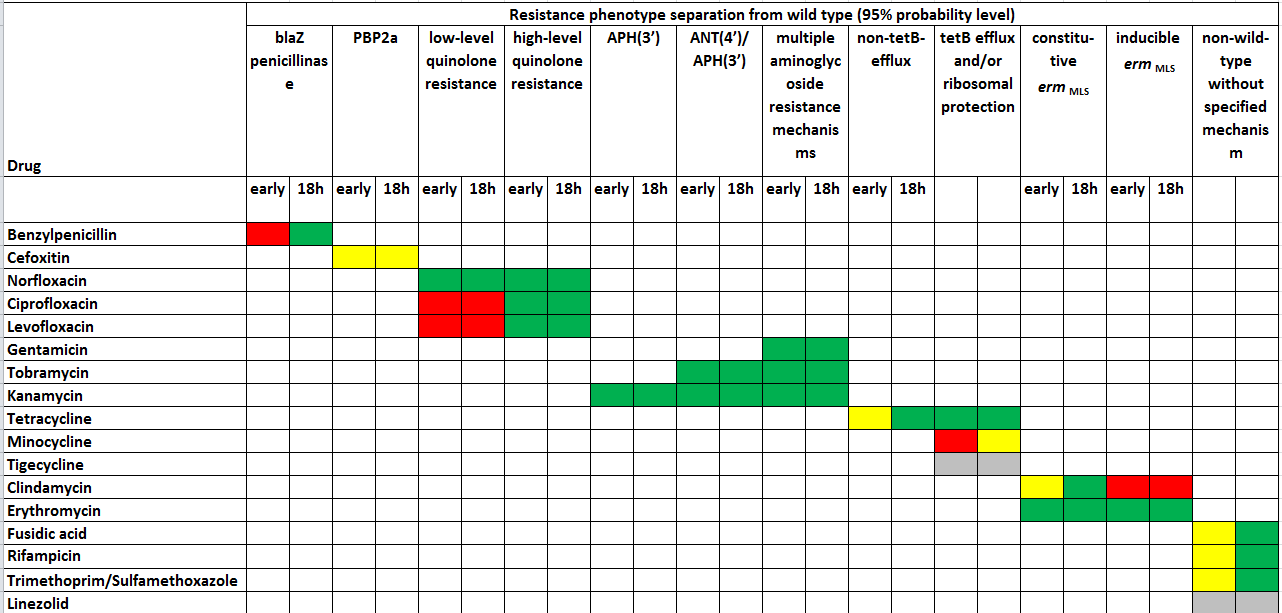
**Tables and Figures**

**Figure 1: Separation of non-wild-type and wild-type populations at early reading times versus 18h of incubation and *Enterobacteriaceae***



Green boxes, wild-type and non-wild-type populations were well separated (i.e. separation with sensitivity and specificity at least 0.95 possible); red boxes, wild-type and non-wild-type populations were not separated (i.e. separation with sensitivity and specificity necessarily at least 0.95 not possible); yellow boxes, separability of wild-type and non-wild-type populations differs among species.

**Figure 2: Separation of non-wild-type and wild-type populations at early reading times versus 18h of incubation and staphylococci**



Separation of non-wild-type and wild-type populations is shown on the 90% probability level, i.e. if …% of populations. Green boxes, wild-type and non-wild-type populations were separated; red boxes, wild-type and non-wild-type populations were not separated; yellow boxes, inter-soecies differences in wild-type and non-wild-type separation; grey boxes, non-wild-type populations not available.

**Table 1: Definitions of resistance phenotypes and wild-types**

|  |  |  |  |
| --- | --- | --- | --- |
| **Drug class/phenotype** | ***Enterobacteriaceae*** | | **staphylococci** |
| **beta-lactams** | see references [13-15](#_ENREF_13), [24](#_ENREF_24) | |  |
| wild-type |  | | benzylpenicillin S AND fuzzy zone edge AND cefoxitin S [25](#_ENREF_25" \o "Weissert,  #2657) |
| ESBL |  | |  |
| AmpC hyperproduction |  | |  |
| aquired AmpC |  | |  |
| carbapenemases |  | |  |
| KPC |  | |  |
| NDM |  | |  |
| OXA-48 |  | |  |
| VIM |  | |  |
| IMP |  | |  |
| other beta-lactamases a) | All non-wild-type, non-ESBL, non-AmpC, non-carbapenemase strains | |  |
| blaZ Penicillinase |  | | benzylpenicillin R or benzylpenicillin S and sharp edge AND cefoxitin S |
| PBP2a |  | | cefoxitin R AND PBP2a agglutination positive and/or *mecA*-PCR positive |
| **Aminoglycosides** [**26**](#_ENREF_26) |  | |  |
| wild-type | gentamicin S; tobramycin S; kanamycin Sb) | | gentamicin S; tobramycin S; kanamycin S |
| APH(3') |  | | gentamicin S; tobramycin S; kanamycin R/I |
| ANT(4')/APH(3') |  | | gentamicin S; tobramycin R; kanamycin R/I |
| ANT(4')/AAC(6')-Ib | gentamicin S; tobramycin R/I; kanamycin R/I b) | |  |
| AAC(2')/AAC(3')-II/-IV | gentamicin R/I; tobramycin R/I; kanamycin S b) | |  |
| multiple mechanisms | gentamicin R/I; tobramycin R/I; kanamycinR/I b) | | gentamicin R; tobramycin R; kanamycin R/I |
| **Quinolones** [**26**](#_ENREF_26) |  | |  |
| wild-type | nalidixic acid S b); norfloxacin S ;ciprofloxacin S | | norfloxacin S; ciprofloxacin R; levofloxacin R |
| low level resistance | nalidixic acid R; norfloxacin S/I; ciprofloxacin S/I | | norfloxacin I/R; ciprofloxacin S/I; levofloxacin S/I |
| high level resistance | nalidixic acid R; norfloxacin R; ciprofloxacin R | | norfloxacin R; ciprofloxacin R; levofloxacin I/R |
| **Trimethoprim/sulfamethoxazole** [**26**](#_ENREF_26) |  | |  |
| wild-type | trimethoprim/sulfamethoxazole S | | |
| non-wild-type | trimethoprim/sulfamethoxazole R | | |
| **Tetracyclines** [**26**](#_ENREF_26) |  |  | |
| wild-type |  | tetracycline S; | |
| efflus PLUS ribosomal protection |  | tetracycline R; minocycline R; tigecycline S | |
| non-tetB-efflux |  | tetracycline R; minocycline S; tigecycline S | |
| non-wild-type (tigecycline) | tigecycline R |  | |
| **MLS-drugs** [**26**](#_ENREF_26) |  |  | |
| wild-type |  | erythromycin S; clindamycin S AND D-shape negative | |
| lnu |  | erythromycin S; clindamycin R | |
| iMLS |  | erythromycin R; clindamycin S AND D-shape positive | |
| Efflux |  | erythromycin R; clindamycin S AND D-shape negative | |
| cMLS |  | erythromycin R; clindamycin R | |
| **Fusidic acid** |  |  | |
| wild-type |  | fusidic acid S b) | |
| non-wild-type |  | fusidic acid R b) | |
| **Oxazolidinones** |  |  | |
| wild-type |  | linezolide S | |
| non-wild-type |  | linezolide R | |
| **Rifampicin** |  |  | |
| wild-type |  | rifampicin S | |
| non-wild-type |  | rifampicin R | |

S, susceptible; I, intermediate; R, resistant. Assignment of phenotypes was conducted based on independent disk diameter measurements generated prior to this study by using the EUCAST recommended disk diffusion method on Müller-Hinton II agar (Beckton-Dickinson, Franklin Lakes, NJ, USA) and with antibiotic discs from i2a (i2a, Montpellier, France) and applying EUCAST CBPs. [19](#_ENREF_19" \o "EUCAST, 2016 #2102) (EUCAST 2016).

a) other beta-lactamases contain e.g. TEM-1/2, SHV-1, OXA-type enzymes that could not be discriminated phenotypically

b) The following breakpoints that are not contained in the EUCAST 2016 version 6.0 of breakpoint tables were applied: Nalidixic acid: S ≥16 mm (EUCAST Clinical Breakpoint Table v. 1.1), fusidic acid: S ≥24 mm (CA-SFM 2012), kanamycin and Enterobacteriaceae: kanamycin: S ≥17 mm (CA-SFM 2012). [22](#_ENREF_22" \o "EUCAST, 2010 #530), [27](#_ENREF_27" \o "CA-SFM, 2012 #2103)

**Table 2: Change of wild-type zone diameters over time**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Drug** | ***E. coli*** | ***K. pneumoniae*** | ***E. cloacae*** | ***S. aureus*** | ***S. epidermidis*** |
|  |  |  |  |  |  |
| Benzylpenicillin |  |  |  | **↑** | **↑** |
| Ampicillin | **↔** | NR | NR |  |  |
| Amoxicillin/clavulanic acid | **↑** | **↔** | NR |  |  |
| Piperacillin/tazobactam | **↑** | **↔** | **↔** |  |  |
| Temocillin | **↔** | **↔** | **↔** |  |  |
| Cefuroxime | **↑** | **↑** | **↑** |  |  |
| Cefoxitin | **↑** | **↔** | NR | **↑** | **↑** |
| Cefpodoxime | **↑** | **↔** | **↑** |  |  |
| Ceftriaxone | **↑** | **↑** | **↑** |  |  |
| Cefepime | **↑** | **↑** | **↑** |  |  |
| Meropenem | **↑** | **↑** | **↑** |  |  |
| Nalidixic acid | **↑** | **↓** | **↓** |  |  |
| Norfloxacin | **↑** | **↑** | **↑** | **↑** | **↑** |
| Ciprofloxacin | **↑** | **↑** | **↑** | **↑** | **↑** |
| Levofloxacin | **↑** | **↑** | **↑** | **↑** | **↑** |
| Gentamicin | **↑** | **↑** | **↑** | **↑** | **↑** |
| Tobramycin | **↑** | **↑** | **↑** | **↑** | **↑** |
| Kanamycin |  |  |  | **↑** | **↑** |
| Tetracycline |  |  |  | **↑** | **↑** |
| Minocycline |  |  |  | **↑** | **↑** |
| Tigecycline | **↑** | **↑** | **↔** | **↑** | **↑** |
| Trimethoprim/sulfamethoxazole | **↓** | **↓** | **↓** | **↑** | **↑** |
| Clindamycin |  |  |  | **↑** | **↑** |
| Erythromycin |  |  |  | **↑** | **↑** |
| Fusidic acid |  |  |  | **↑** | **↑** |
| Rifampicin |  |  |  | **↑** | **↑** |
| Linezolid |  |  |  | **↑** | **↑** |

Arrows indicate the relative direction of zone diameter changes over time:

↑ increase; ↔ stable; ↓ decrease; NR, natural resistance