Enterococcus faecium: from evolutionary insights to practical interventions

Zhou, Xue Wei

Link to publication in University of Groningen/UMCG research database

Diagnostic evasion of highly-resistant microorganisms: a critical factor in nosocomial outbreaks

6

X. Zhou[1], A.W. Friedrich[1], E. Bathoorn[1]

[1]University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, The Netherlands

Corresponding author: Alexander W. Friedrich. Address: Hanzeplein 1 EB80, 9713GZ Groningen, the Netherlands.

Tel: +31 50 3613480; Fax: +31 50 3619105; Email: alex.friedrich@umcg.nl

ABSTRACT

Highly resistant microorganisms (HRMOs) may evade screening strategies used in routine diagnostics. Bacteria that have evolved to evade diagnostic tests may have a selective advantage in the nosocomial environment. Evasion of resistance detection can result from the following mechanisms: low-level expression of resistance genes not resulting in detectable resistance, slow growing variants, mimicry of wild-type-resistance and resistance mechanisms that are only detected if induced by antibiotic pressure.

We reviewed reports on hospital outbreaks in the Netherlands over the past 5 years. Remarkably, many outbreaks including major nation-wide outbreaks were caused by microorganisms able to evade resistance detection by diagnostic screening tests. We describe various examples of diagnostic evasion by several HRMOs and discuss this in a broad and international perspective.

The epidemiology of hospital-associated bacteria may strongly be affected by diagnostic screening strategies. This may result in an increasing reservoir of resistance genes in hospital populations that is unnoticed. The resistance elements may horizontally transfer to hosts with systems for high-level expression, resulting in a clinically significant resistance problem.

We advise to communicate the identification of highly resistant microorganisms that evade diagnostics within national and regional networks. Such signaling networks may prevent for inter-hospital outbreaks, and allow for collaborative development of adapted diagnostic tests.

INTRODUCTION

Diagnostic screening provides hospitals a level of immunity to antibiotic resistance. When highly resistant microorganisms (HRMOs) are detected, transmission can be limited by treating the patient with isolation precautions. In addition, the carriage of HRMOs can be suppressed by antibiotic treatment or, in case of methicillin resistant *Staphylococcus aureus* (MRSA), even be eradicated. If the introduction of HRMOs in hospitals remains undetected, these bacteria can disseminate from patient-to-patient, and the mobile genetic elements carrying resistance genes can horizontally transfer from species-to-species. Thus, the epidemiology of nosocomial resistance may strongly be affected by our diagnostic screening strategies. Moreover, we postulate that evasion of diagnostic resistance screening could be considered as a critical factor for infection of hospitals with antibiotic resistance elements, similar to the concept that immune evasion is a critical factor of pathogens to infect the human host.

The Netherlands is a high-resource country. Surveillance on HRMOs is extensive in Dutch hospitals. For this, the Dutch situation is very proficient to observe effects of diagnostic screening on the characteristics of HRMOs that cause nosocomial outbreaks. The Dutch Society for Medical Microbiology (NVMM) provides guidelines for the detection of HRMOs [1]. For the detection of HRMOs such as carbapenemase-producing Enterobacteriaceae (CPE), vancomycin-resistant enterococci (VRE), extended spectrum beta-lactamase (ESBL) - producing bacteria and MRSA, selective broth and/or selective media are used. Nosocomial outbreaks with HRMO are reported to 'Hospital Acquired Infection and Antimicrobial Resistance Monitoring Group" and the reports are communicated to clinical microbiologists. We searched these reports for outbreaks with micro-organisms harboring resistance mechanisms that were able to evade detection by routine diagnostics. In addition, we evaluated data from our hospital, and searched literature for outbreaks to assess the importance of diagnostic evasion. We here present the most explicit examples of CPE, VRE, ESBL-producing bacteria and MRSA outbreaks caused by isolates harboring diagnostic-evasive resistance mechanisms.

Diagnostic evasion by CPEs

In the Netherlands, the national laboratory guideline recommends the following screening strategy for the detection of highly resistant microorganisms: a screening step, a genotypic confirmation step and an optional phenotypic confirmation step [1, 2]. According to this guideline, *Enterobacteriaceae* with an MIC for meropenem ≥0.50 mg/L, or imipenem ≥2.0 mg/L should be evaluated by molecular tests for carbapenemase gene detection. Optional

phenotypic tests, which include the modified Hodge test, and tests based on inhibition of metallo-betalactamases by EDTA, and Class A carbapenemases by phenyl-boronic acid, may be used if genotypic confirmatory tests are not immediately available. Newer tests for non-genotypic detection of CPE include the carba-NP test, carbapenem-inactivation method (CIM-test), and immunochromotographic tests [3-6]. Genotypic confirmation comprises PCR and sequence based methods. Next-gen-sequencing facilities are increasingly accessible for routine diagnostic laboratories. This allows whole-genome sequence-based carbapenemase gene detection. In addition, specific primer/probe combinations for unique markers of an outbreak strains may be designed for high-throughput diagnostics to control outbreaks [7].

Despite this huge arsenal of CPE-detection methods, CPEs are still able to evade our diagnostic screening strategies. In the Netherlands, an inter-hospital outbreak with OXA-48-producing *Enterobacteriaceae* from 2009-2011 has been reported [8]. The outbreak had been uncontrolled for 2 years. The plasmids carrying bla_{OXA-48} had disseminated to 15 (sub)-species. Predominantly OXA-48-producing *E. coli* and *K. pneumoniae* isolates were detected. Heterogeneity in resistance to carbapenems within, and across the OXA-48-producing species was observed. All OXA-48-producing *E. coli* isolates had meropenem MICs of <1 mg/L, a concentration that is commonly used in screening plates, whereas the meropenem susceptibility breakpoint for meropenem is 2mg/L according to EUCAST [9]. In addition, if the OXA-48 was not co-expressed with an ESBL gene, no hydrolysis of 3th generation cephalosporins was detected in the majority of isolates. These diagnostic stealth-features have undoubtedly contributed to the magnitude of this outbreak.

The emergence and spread of OXA-48 producing CPEs have been reported in several countries in Europe [10]. The outbreaks concerned predominantly *K. pneumoniae* clones. A successful *K. pneumoniae* clone carrying OXA-48 is ST 11, reported in many countries [11], amongst others in Greece [12], Spain [13] and Belgium [14]. Other clones associated with OXA-48 are ST14, ST15, ST101, SST 147 and ST405 [11, 13, 15]. In a Belgian multi-center study, less than 50% of CPEs were carbapenem non-susceptible [14].

Given the fact that OXA-48 is difficulty to detect, there is a need to adapt surveillance strategies to detect CPEs. The EUCAST-guideline advises to screen for CPE if isolates have a MIC to meropenem >0.12 mg/L [9]. Unfortunately, widely used automated susceptibility testing (AST) systems do not detect MICs below 0.5 mg/L. The meropenem MIC distribution of OXA-48-producing *Enterobacteriaceae*, however, shows a peak at MIC=0.25 mg/L [16]. These isolates will remain undetected if screened by AST only.

When using screening cut-off MICs for CPE detection, which are lower than the susceptibility cut-offs, the sensitivity is still just 80% [17]. Mainly OXA-48-, and some VIM-producers would remain undetected using meropenem screening cut-offs. Since carbapenem-resistant isolates are usually send to reference centers for CPE detection, this may result in an underestimation of true prevalence numbers [18]. In our hospital, we use both culture on screening agars and carbapenemase gene detection directly on rectum samples in patients with a high risk on CPE-carriage to increase the sensitivity of surveillance cultures [19]. Direct screening of rectal swabs for carbapenemases by real-time PCR performed on enrichment broth showed a higher sensitivity than culturing on selective agar plates [20]. However, relying on genotypic tests alone may also be a pitfall. For instance, molecular panels for detection of CPE may have a limited number of carbapenemase gene targets. CPEs that are not detected by the panel may have an evolutionary advantage caused by the limitations of this diagnostic method.

Diagnostic evasion by VRE

A second example of successful diagnostic evasion by HRMOs is the nationwide emergence of nosocomial outbreaks with vancomycin-resistant *Enterococci* (VRE) in the Netherlands. In the period 2012-2014, 26 outbreaks with VRE have been reported, including reports of local and inter-hospital transmissions [21]. Outbreaks predominantly occurred with VanA- and VanB-type *Enterococcus faecium*, that confer resistance to glycopeptides. *VanB* VRE can easily remain undetected by culturing in routine diagnostics. In addition to the fact that fecal VRE carriage often is detected in very low amounts, vancomycin resistance in *vanB* VRE is not always expressed. These diagnostic challenges have been an important factor in the ongoing transmission of VRE in hospitals in the Netherlands. Several phenotypic screening methods, such as the use of chromogenic agars, have been suggested to identify *vanB* VRE with varying vancomycin MICs [22]. However, VRE suspected colonies growing on Chrome-agars may test vancomycin susceptible in routine AST systems despite positive genotypic confirmation of *vanB*. This could lead to an unnoticed and uncontrolled spread of *vanB* VRE.

In our hospital, patients are screened on a PCR-based method for VRE on admission at the intensive care unit and if patients are transferred from or recently have been admitted in another hospital in the Netherlands or a foreign hospital. If an unexpected VRE case is found, screening is performed in those patients who are at risk of VRE transmission.

We have reviewed VRE data from 2013-2016 in our own hospitals. We searched for all VRE positive patients and selected their first VRE sample. A total of 106 patients were found, all isolates were *vanB E. faecium*. Of these *vanB* VREs, 26 isolates (24.5%) were tested

vancomycin-susceptible by Vitek2 (bioMérieux) according to the EUCAST susceptibility breakpoint of ≤4mg/L [23]. Vancomycin 5 µg paperdisks (Becton Dickenson) were used to phenotypically detect the resistance mechanism, which showed an hazy edge also in the *vanB* positive vancomycin-susceptible isolates. Of these 26 isolates, 24 were outbreak related (92.3%). The two non-outbreak related isolates in the vancomycin-susceptible group were found in a patient transferred from another Dutch hospital and in a patient transferred from a foreign hospital. The other 80 isolates (75.5%) were tested resistant to vancomycin. Of these, 65 isolates (81.3%) were outbreak related (Figure 1). The 15 non-outbreak related isolates in this group were from the surveillance cultures of patients transferred from hospitals abroad (n=1), patients transferred from other Dutch hospitals (n=2), in patients admitted to the ICU (n=8), and in clinical samples (n=4). Noticeably, among these 80 patients with vancomycin-resistant *vanB*, we also detected *vanB* positive vancomycin-susceptible *E. faecium* isolates in follow-up samples from 13 patients. These results are in line with reports in literature. A VRE outbreak in a neonatal ICU in Germany has been reported, in which even 55% of the *vanB* positive VRE isolates were tested vancomycin susceptible [24]. These data show the possible pitfalls in detecting *vanB* VRE in a significant population when only using phenotypic screening tests.

Figure 1: Number of first VRE (all *vanB E. faecium*) isolates from patients during 2013-2016 and their corresponding MIC values. The dashed line represents the vancomycin susceptibly breakpoint of 4mg/L.
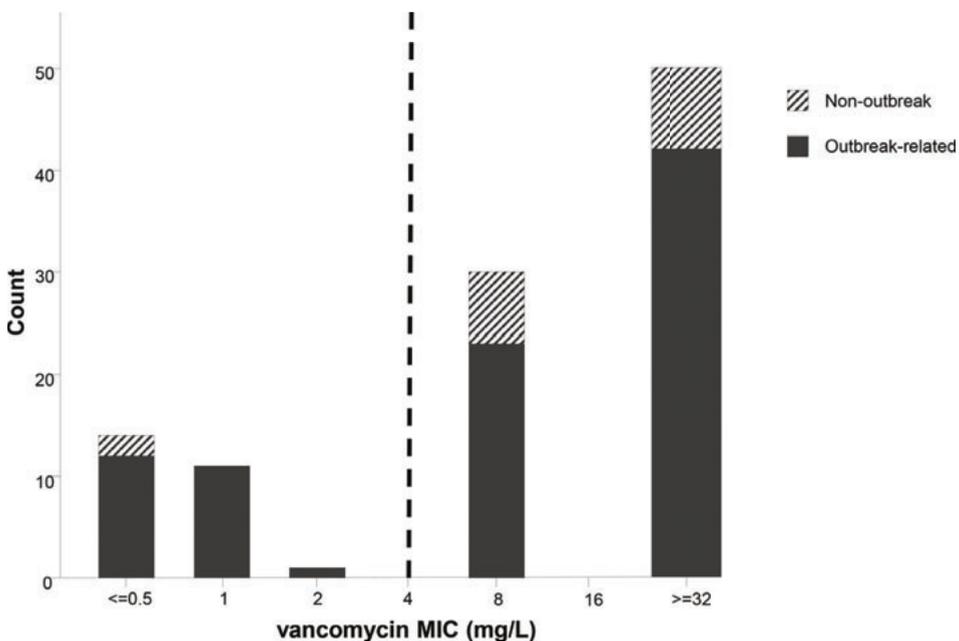
Pitfalls in detecting *vanA* VRE have been described due to an altered phenotype of *vanA* VRE. The expression of teicoplanin resistance can be heterogenous conferring into a VanB phenotype [25]. Moreover, isolates can even test vancomycin susceptible due to a silenced *vanA* gene which can easily lead to uncontrolled outbreaks [26, 27].

In a multicenter study the EUCAST disk diffusion method performed significantly better than the Vitek2 system for the detection of clinical enterococci isolates with low and medium level vancomycin resistance [28]. For rapid detection of VRE carriage, diagnostic strategies using selective enrichment broths and molecular detection can be used to increase the sensitivity of diagnostic procedures [29]. Based on above findings, genotypic testing of invasive vancomycin-susceptible enterococci by PCR can be advised. All three diagnostic strategies are being used in our routine diagnostic laboratory.

Diagnostic evasion by MRSA

To detect MRSA carriage, the Dutch laboratory guideline recommends to take samples of the throat, nose, and perineum [1]. Additional body sites should be sampled depending on clinical signs such as wounds, productive cough, skin lesions, or indwelling catheters. To optimize the sensitivity of the cultures, incubation in relatively non-selective enrichment salt-only broths is recommended, followed by culturing for 48 hours on selective MRSA screening agars. Additional rapid molecular test are recommended in case of urgency.

In the Netherlands, patients with risk factors for MRSA–carriage such as recent hospitalization abroad, or *farm workers* at pig *farms*, cattle *farms,* or poultry *farms* are treated upon admission in strict isolation until rapid PCR-based diagnostics are negative. In case of MRSA carriage, patients are treated in isolation and MRSA eradication therapy can be started. This is known as the search and destroy policy [30]. However, PCR-based diagnostics for screening alone would not detect all cases of MRSA-carriage. In a meta-analysis, a sensitivity of 92.5% for the overall pooled PCR estimate has been reported, with a high level of heterogeneity among the studies [31]. PCR-based false negative MRSA results are in our experience usually in patients with a low-level carriage of MRSA. In these cases, culture on chromogenic agar after incubation in broth is more sensitive. In our hospital we use the GeneXpert, an automated PCR-based method to detect MRSA. The lower detection limit for the Xpert MRSA SA nasal assay is about 70 colony forming units (CFU)/sample according to the manufacturer.

A second reason for failure to detect MRSA is that sporadic Staphylococcal Cassette Chromosome mec (SCCmec)-cassette subtypes, which are a common target in commercial

6

tests, may not be detected by PCR. There is a high diversity in SCCmec-cassettes: already 11 SCCmec-types and numerous subtypes have been designated [32]. The detection of SCCmec by PCR-based tests is still improving, and the coverage has expanded over the recent years. However, since there may be shifts in common lineages, we should be aware of sporadic nosocomial MRSA that may emerge as successful clones, and are undetectable by commercial tests [33]. Variety in the *mecA/mecC* target may also result in failure of MRSA detection by PCR. For instance, MRSAs with the divergent homologue *mecA* ($mecA_{LGA251}$) would not be detected by the Xpert MRSA assays [34].

False-negative PCR results may have a considerable impact in hospitals. Since patients are discharged from strict isolation after negative PCR results, the isolate has an opportunity to spread until the MRSA is identified by culture and the patient is in strict isolation again. To prevent further spread, contact investigations among patients in the same room and health care workers are performed in these cases in the Netherlands. Since PCR-based detection is not reliable in screening for such isolates, the investigation of contacts has to be performed by culture, which delays the time to detection of secondary transmissions.

Not only PCR-based diagnostics, but also culture-based detection may be evaded by MRSA. In 2014, clinical microbiologists were alerted by a report of the monitoring group on an outbreak with a MRSA strain that could easily be missed by routine diagnostics. Although the numbers of transmissions were largely reduced, total control of the outbreak was difficult due to detection problems using conventional culturing. The *mecA*-positive isolate was difficult to culture as the oxacillin MIC was low, ranging from 0.5 to 6.0 µg/mL. Growth on ChromID™ MRSA agar (bioMérieux) plates was strongly inhibited. We tested the outbreak isolate in our own laboratory and found a more then 10-fold decrease in colony numbers if cultured on ChromID™ MRSA plates compared to blood agar, resulting in a detection limit on ChromID™ MRSA below 0.5×10^3 colonies/100µL. Molecular testing and prolonged subculturing in broths was advised to detect this isolate.

Diagnostic evasion by ESBL

ESBL-detection can be complicated in natural AmpC-producers such as *Citrobacter freundii, Enterobacter* spp., *Hafnia alvei, Morganella morganii, Serratia* spp. and *Providencia* spp, since it mimics their natural resistance pattern. Antibiotics can select for mutants with derepressed AmpC expression, resulting in resistance to cephalosporins during therapy. Thus, antibiotic treatment with cephalosporins is not recommended [35]. Presence of natural AmpC alone is no condition for HRMO and infection prevention measures.

However, in 2015, several outbreaks in various hospitals in the Netherlands were reported with natural AmpC-producing *Enterobacteriaceae* that acquired additional ESBL genes. This has no consequences for antibiotic therapy choices, however, infection prevention measures need to be taken.

This combined "AmpC-plus-ESBL" phenotype is difficult to distinguish from derepressed-AmpC wild-type resistance. The Dutch laboratory guideline recommend to use cefotaxim and/or ceftazidim to screen for ESBLs with cut-off MIC values for both cephalosporins of >1 mg/L. This screening strategy is also for *Enterobacteriaceae* with natural AmpCs. This leads to many false positive results due to derepressed AmpCs. Phenotypic confirmation based on inhibition ESBL activity by clavulanic acid or cefepime hydrolysis by disk diffusion, Etest or broth microdilution methods is recommended.

Natural AmpC-producing *Enterobacteriaceae* that acquired additional ESBL genes are common in Dutch nosocomial isolates. *Citrobacter freundii* and *Enterobacter cloacae* showed the highest percentages of confirmed ESBL co-producers: 3% of *Citrobacter freundii* (total n=9.432), and 2% of *Enterobacter cloacae* (n=28.027) were recorded by the Dutch national antibiotic resistance surveillance system (ISIS-AR). Microbiologist were explicitly warned for outbreaks with these difficult to detect HRMOs in a report by the monitoring group.

The substantial presence of ESBLs in *Enterobacteriaceae* with natural AmpCs has been underlined in an Asian study [36]. The ESBLs confer additional resistance to fourth generation cephalosporins, compared to the natural broad-spectrum AmpCs. These isolate may represent a hidden reservoir of ESBL-carrying plasmids, which can transfer across species. Numerous outbreaks with ESBL natural AmpC producers have been reported in international literature [37]. Since resistance to 3th generation cephlosporins is very common in natural AmpC producers that do not carry ESBLs [38], the dissemination of ESBL-carrying isolates in hospitals may remain unnoticed.

6

Implications and future directions

We observed that highly-resistant microorganisms adapt to evade screening strategies. One can consider this process as a prey that evolves to escape from predators. Microbiologists, in their evolutionary role as predators hunting for HRMOs, also have to keep on innovating to update the detection strategies for these micro-organisms that are trying to evade. This may result in an arms race. In evolutionary biology, such an arms race is known as the Red Queens hypothesis [39]. The name of the theory is based on a quote from Lewis Carroll's *Through the Looking-Glass*: "Now, here, *you* see, it takes all the *running you* can do,

to *keep* in the *same* place. If *you* want to *get* somewhere else, *you* must *run* at least twice as *fast* as that!".

To run twice at fast, communication within networks of health care professionals is crucial. In our perspective, we described examples of how Dutch clinical microbiologist were alarmed by a national monitoring group on successful HRMOs that evade routine screening tests. Specific recommendations to adjust diagnostic strategies to detect these pathogens were provided. Additionally, rapid communication within regional networks is of utmost importance. Inter-hospital patient traffic is highest between hospitals in the same regions. As a consequence, hospitals within the same region are at immediate risk of introduction of HRMOs that evade diagnostics and cause outbreaks. We recommend to identify your region of hospitals that are most connected by patient traffic, and set-up communication networks to alarm for difficult to detect HRMO's. Experiences and adjusted diagnostic screening tests should be shared within these networks. Such a regional approach has successfully been applied in the control of MRSA in the Dutch-German cross-border region [40].

We should be aware of the impact of our diagnostics on the introduction and dissemination of resistance elements in our hospitals. The Government of the Netherlands has a national and international mission to combat antimicrobial resistance (AMR). Therefore the NVMM has composed a vision document to maintain the low prevalence of CPE in the Netherlands [41]. By taking CPE as a biological indicator, it is implicitly assumed that other HRMOs will be included in the combat of AMR. To realize the goals, it is of utmost importance that diagnostic methods are continuously innovated and used.

We are aware that optimizing diagnostic screening will increase costs. On the other hand, our examples have shown that failure of detection by routine diagnostics may lead to uncontrolled outbreaks. These outbreaks can lead to enormous financial expenses; costs may rise up to €1,369 per patient per day [42]. Moreover, detection of HRMO carriage allows for directed antibiotic treatment of patients developing infections by these HRMOs.

Cost reductions in innovation of diagnostics for screening purposes are foretold to result in nosocomial outbreaks with HRMOs evading our screenings methods. We would be outsmarted by prokaryotes.

REFERENCES

1. Working group members NVMM: NVMM Guideline Laboratory detection of highly resistant microorganisms (HRMO). 2012, version 2:.

2. Cohen Stuart J, Leverstein-Van Hall MA, Dutch Working Party on the Detection of Highly Resistant Microorganisms: Guideline for phenotypic screening and confirmation of carbapenemases in Enterobacteriaceae. Int J Antimicrob Agents 2010, 36(3):205-210.

3. Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V, European Network on Carbapenemases: Identification and screening of carbapenemase-producing Enterobacteriaceae. Clin Microbiol Infect 2012, 18(5):432-438.

4. Literacka E, Herda M, Baraniak A, Zabicka D, Hryniewicz W, Skoczynska A, Gniadkowski M: Evaluation of the Carba NP test for carbapenemase detection in Enterobacteriaceae, Pseudomonas spp. and Acinetobacter spp., and its practical use in the routine work of a national reference laboratory for susceptibility testing. Eur J Clin Microbiol Infect Dis 2017, .

5. van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM: The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. PLoS One 2015, 10(3):e0123690.

6. Dortet L, Jousset A, Sainte-Rose V, Cuzon G, Naas T: Prospective evaluation of the OXA-48 K-SeT assay, an immunochromatographic test for the rapid detection of OXA-48-type carbapenemases. J Antimicrob Chemother 2016, 71(7):1834-1840.

7. Deurenberg RH, Bathoorn E, Chlebowicz MA, Couto N, Ferdous M, Garcia-Cobos S, Kooistra-Smid AM, Raangs EC, Rosema S, Veloo AC, Zhou K, Friedrich AW, Rossen JW: Application of next generation sequencing in clinical microbiology and infection prevention. J Biotechnol 2017, 243:16-24.

8. Dautzenberg MJ, Ossewaarde JM, de Kraker ME, van der Zee A, van Burgh S, de Greeff SC, Bijlmer HA, Grundmann H, Cohen Stuart JW, Fluit AC, Troelstra A, Bonten MJ: Successful control of a hospital-wide outbreak of OXA-48 producing Enterobacteriaceae in the Netherlands, 2009 to 2011. Euro Surveill 2014, 19(9):20723.

9. EUCAST subcommittee for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance: EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. July 2017, :.

10. Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasevic AT, Canton R, Carmeli Y, Friedrich AW, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Nordmann P, Poirel L, Rossolini GM, Seifert H, Vatopoulos A, Walsh T, Woodford N, Monnet DL, European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) Working Group: Occurrence of carbapenemase-producing Klebsiella pneumoniae and Escherichia coli in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. Lancet Infect Dis 2017, 17(2):153-163.

11. Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH: Global Dissemination of Carbapenemase-Producing Klebsiella pneumoniae: Epidemiology, Genetic Context, Treatment Options, and Detection Methods. Front Microbiol 2016, 7:895.

12. Voulgari E, Zarkotou O, Ranellou K, Karageorgopoulos DE, Vrioni G, Mamali V, Themeli-Digalaki K, Tsakris A: Outbreak of OXA-48 carbapenemase-producing Klebsiella pneumoniae in Greece involving an ST11 clone. J Antimicrob Chemother 2013, 68(1):84-88.

13. Oteo J, Ortega A, Bartolome R, Bou G, Conejo C, Fernandez-Martinez M, Gonzalez-Lopez JJ, Martinez-Garcia L, Martinez-Martinez L, Merino M, Miro E, Mora M, Navarro F, Oliver A, Pascual A, Rodriguez-Bano J, Ruiz-Carrascoso G, Ruiz-Garbajosa P, Zamorano L, Bautista V, Perez-Vazquez M, Campos J, GEIH-GEMARA (SEIMC) and REIPI: Prospective multicenter study of carbapenemase-producing Enterobacteriaceae from 83 hospitals in Spain reveals high in vitro susceptibility to colistin and meropenem. Antimicrob Agents Chemother 2015, 59(6):3406-3412.

6

14. De Laveleye M, Huang TD, Bogaerts P, Berhin C, Bauraing C, Sacre P, Noel A, Glupczynski Y, multicenter study group: Increasing incidence of carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in Belgian hospitals. Eur J Clin Microbiol Infect Dis 2017, 36(1):139-146.

15. Liapis E, Pantel A, Robert J, Nicolas-Chanoine MH, Cavalie L, van der Mee-Marquet N, de Champs C, Aissa N, Eloy C, Blanc V, Guyeux C, Hocquet D, Lavigne JP, Bertrand X, ONERBA: Molecular epidemiology of OXA-48-producing Klebsiella pneumoniae in France. Clin Microbiol Infect 2014, 20(12):O1121-3.

16. Fattouh R, Tijet N, McGeer A, Poutanen SM, Melano RG, Patel SN: What Is the Appropriate Meropenem MIC for Screening of Carbapenemase-Producing Enterobacteriaceae in Low-Prevalence Settings? Antimicrob Agents Chemother 2015, 60(3):1556-1559.

17. Huang TD, Poirel L, Bogaerts P, Berhin C, Nordmann P, Glupczynski Y: Temocillin and piperacillin/tazobactam resistance by disc diffusion as antimicrobial surrogate markers for the detection of carbapenemase-producing Enterobacteriaceae in geographical areas with a high prevalence of OXA-48 producers. J Antimicrob Chemother 2014, 69(2):445-450.

18. Kaase M, Schimanski S, Schiller R, Beyreiss B, Thurmer A, Steinmann J, Kempf VA, Hess C, Sobottka I, Fenner I, Ziesing S, Burckhardt I, von Muller L, Hamprecht A, Tammer I, Wantia N, Becker K, Holzmann T, Furitsch M, Volmer G, Gatermann SG: Multicentre investigation of carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in German hospitals. Int J Med Microbiol 2016, 306(6):415-420.

19. Bathoorn E, Friedrich AW, Zhou K, Arends JP, Borst DM, Grundmann H, Rossen JW: Latent introduction to the Netherlands of multiple antibiotic resistance including NDM-1 after hospitalisation in Egypt, August 2013. Euro Surveill 2013, 18(42):20610.

20. Singh K, Mangold KA, Wyant K, Schora DM, Voss B, Kaul KL, Hayden MK, Chundi V, Peterson LR: Rectal screening for Klebsiella pneumoniae carbapenemases: comparison of real-time PCR and culture using two selective screening agar plates. J Clin Microbiol 2012, 50(8):2596-2600.

21. van der Bij AK, Kardamanidis K, Frakking FN, Bonten MJ, Signaleringsoverleg Ziekenhuisinfecties en Antimicrobiele Resistentie: Nosocomial outbreaks and resistant microorganisms. Ned Tijdschr Geneeskd 2015, 159:A8585.

22. Klare I, Fleige C, Geringer U, Witte W, Werner G: Performance of three chromogenic VRE screening agars, two Etest((R)) vancomycin protocols, and different microdilution methods in detecting vanB genotype Enterococcus faecium with varying vancomycin MICs. Diagn Microbiol Infect Dis 2012, 74(2):171-176.

23. EUCAST clinical breakpoints

24. Werner G, Klare I, Fleige C, Geringer U, Witte W, Just HM, Ziegler R: Vancomycin-resistant vanB-type Enterococcus faecium isolates expressing varying levels of vancomycin resistance and being highly prevalent among neonatal patients in a single ICU. Antimicrob Resist Infect Control 2012, 1(1):21.

25. Park IJ, Lee WG, Shin JH, Lee KW, Woo GJ: VanB phenotype-vanA genotype Enterococcus faecium with hetero-geneous expression of teicoplanin resistance. J Clin Microbiol 2008, 46(9):3091-3093.

26. Gagnon S, Levesque S, Lefebvre B, Bourgault AM, Labbe AC, Roger M: vanA-containing Enterococcus faecium susceptible to vancomycin and teicoplanin because of major nucleotide deletions in Tn1546. J Antimicrob Chemother 2011, 66(12):2758-2762.

27. Sivertsen A, Pedersen T, Larssen KW, Bergh K, Ronning TG, Radtke A, Hegstad K: A Silenced vanA Gene Cluster on a Transferable Plasmid Caused an Outbreak of Vancomycin-Variable Enterococci. Antimicrob Agents Chemother 2016, 60(7):4119-4127.

28. Hegstad K, Giske CG, Haldorsen B, Matuschek E, Schonning K, Leegaard TM, Kahlmeter G, Sundsfjord A, Nor-dicAST VRE Detection Study Group: Performance of the EUCAST disk diffusion method, the CLSI agar screen

method, and the Vitek 2 automated antimicrobial susceptibility testing system for detection of clinical isolates of Enterococci with low- and medium-level VanB-type vancomycin resistance: a multicenter study. J Clin Microbiol 2014, 52(5):1582-1589.

29. Zhou X, Arends JP, Kampinga GA, Ahmad HM, Dijkhuizen B, van Barneveld P, Rossen JW, Friedrich AW: Evaluation of the Xpert vanA/vanB assay using enriched inoculated broths for direct detection of vanB vancomycin-resistant Enterococci. J Clin Microbiol 2014, 52(12):4293-4297.

30. Wertheim HF, Vos MC, Boelens HA, Voss A, Vandenbroucke-Grauls CM, Meester MH, Kluytmans JA, van Keulen PH, Verbrugh HA: Low prevalence of methicillin-resistant Staphylococcus aureus (MRSA) at hospital admission in the Netherlands: the value of search and destroy and restrictive antibiotic use. J Hosp Infect 2004, 56(4):321-325.

31. Luteijn JM, Hubben GA, Pechlivanoglou P, Bonten MJ, Postma MJ: Diagnostic accuracy of culture-based and PCR-based detection tests for methicillin-resistant Staphylococcus aureus: a meta-analysis. Clin Microbiol Infect 2011, 17(2):146-154.

32. Liu J, Chen D, Peters BM, Li L, Li B, Xu Z, Shirliff ME: Staphylococcal chromosomal cassettes mec (SCCmec): A mobile genetic element in methicillin-resistant Staphylococcus aureus. Microb Pathog 2016, 101:56-67.

33. Kinnevey PM, Shore AC, Brennan GI, Sullivan DJ, Ehricht R, Monecke S, Coleman DC: Extensive genetic diversity identified among sporadic methicillin-resistant Staphylococcus aureus isolates recovered in Irish hospitals between 2000 and 2012. Antimicrob Agents Chemother 2014, 58(4):1907-1917.

34. Garcia-Alvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RL, Larsen AR, Skov RL, Peacock SJ, Maskell DJ, Holmes MA: Meticillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 2011, 11(8):595-603.

35. Livermore DM, Brown DF, Quinn JP, Carmeli Y, Paterson DL, Yu VL: Should third-generation cephalosporins be avoided against AmpC-inducible Enterobacteriaceae? Clin Microbiol Infect 2004, 10(1):84-85.

36. Choi SH, Lee JE, Park SJ, Kim MN, Choo EJ, Kwak YG, Jeong JY, Woo JH, Kim NJ, Kim YS: Prevalence, microbiology, and clinical characteristics of extended-spectrum beta-lactamase-producing Enterobacter spp., Serratia marcescens, Citrobacter freundii, and Morganella morganii in Korea. Eur J Clin Microbiol Infect Dis 2007, 26(8):557-561.

37. Mezzatesta ML, Gona F, Stefani S: Enterobacter cloacae complex: clinical impact and emerging antibiotic resistance. Future Microbiol 2012, 7(7):887-902.

38. Jacobson KL, Cohen SH, Inciardi JF, King JH, Lippert WE, Iglesias T, VanCouwenberghe CJ: The relationship between antecedent antibiotic use and resistance to extended-spectrum cephalosporins in group I beta-lactamase-producing organisms. Clin Infect Dis 1995, 21(5):1107-1113.

39. Castrodeza C: Non-progressive evolution, the Red Queen hypothesis, and the balance of nature. Acta Biotheor 1979, 28(1):11-18.

40. Jurke A, Kock R, Becker K, Thole S, Hendrix R, Rossen J, Daniels-Haardt I, Friedrich A: Reduction of the nosocomial meticillin-resistant Staphylococcus aureus incidence density by a region-wide search and follow-strategy in forty German hospitals of the EUREGIO, 2009 to 2011. Euro Surveill 2013, 18(36):pii=20579.

41. Jan Kluytmans, Greet Vos, Christina Vandenbroucke-Grauls, Alexander W. Friedrich, Andreas Voss and Marc Bonten: Nederland CRE-groen: "Optimalisatie van de beheersing van antimicrobiële resistentie door regionale netwerkvorming". Visiedocument NVMM. 6 oktober 2015, :.

42. Dik JW, Dinkelacker AG, Vemer P, Lo-Ten-Foe JR, Lokate M, Sinha B, Friedrich AW, Postma MJ: Cost-Analysis of Seven Nosocomial Outbreaks in an Academic Hospital. PLoS One 2016, 11(2):e0149226.

6