APPENDIX A – REFERENCES

Chapter 1 - General introduction

Breil B, Fritz F, Thiemann V, Dugas M. Mapping turnaround times (TAT) to e generic timeline: a systematic review of TAT definitions in clinical domains. BMC Medical Informatics Decision Making 2011;11:34.

Carmeli Y, Eliopoulos G, Mozaffari E, Samore M. Health and economic outcomes of vancomycin-resistant enterococci. Arch Intern med 2002;162:2223-8.

Cosgrove SE, Qi Y, Kaye KS, Harbarth S, Karchmer AW, Carmeli Y. The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay, and hospital charges. Infect Control Hosp Epidemiol 2005;26:166-74.

Creamer E, Dolan A, Sherlock O, Thomas T, Walsh J, Moore J, Smyth E, O'Neill E, Shore A, Sullivan D, Rossney AS, Cunney R, Coleman D, Humpreys H. The effect of rapid screening for methicillin-resistant *Staphylococcus aureus* (MRSA) on the identification and earlier isolation of MRSA-positive patients. Infect Control Hosp Epidemiol 2010;31:374-81.

Dutch Workingparty on Infection Prevention. Measures to prevent transmission of highly resistant microorganisms (HRMO). 2005. Available at: http://www.wip.nl.

Dutch Workingparty on Infection Prevention. MRSA hospital. 2007. Available at: http://www.wip.nl.

EARSS. EARSS annual report 2008. Available at: http://www.rivm.nl/earss.

EUCAST. Breakpoint tables for interpretation of MICs and zone diameters. Version 2.0, 2012. Available at: http://www.eucast.org.

Galar A, Leiva J, Espinosa M, Guillén-Grima F, Hernáez S, Yuste JR. Clinical and economic evaluation of the impact of rapid microbiological diagnostic testing. J Infect 2012;65:302-9.

Hawkins RC. Laboratory turnaround time. Clin Biochem Rev 2007;28:179-94.

Howanitz JH, Howanitz PJ. Timeliness as a quality attribute and strategy. Am J Clin Pathol 2001;116:311-5.

Kluytmans-VandenBergh MFQ, Kluytmans JAJW, Voss A. Dutch guideline for preventing nosocomial transmission of highly resistant microorganisms (HRMO). Infection 2005;33:309-13.

Leclercq R, Cantón R, Brown DFJ, Giske CG, Heisig P, MacGowan AP, Mouton JW, Nordmann P, Rodloff AC, Rossolini GM, Soussy CJ, Steinbakk M, Winstanley TG, Kahlmeter G. EUCAST expert rules in antimicrobial susceptibility testing. CMI 2011;doi 10.1111/j.1469-0691.2011.03703.x.

Mauldin PD, Salgado CD, Hansen IS, Durup DT, Bosso JA. Attributable hospital cost and length of stay associated with health care-associated infection caused by antibiotic-resistant gram-negative bacteria. Antimicrob Agents Chemother 2010;54:109-15.

Metan G, Zarakolu P, Unal S. Rapid detection of antibacterial resistance in emerging Gram-positive cocci. J Hosp Infect 2005;61:93-9.

Netherlands Society for Medical Microbiology (NVMM). NVMM richtlijn: Detectie van meticilline-resistente *Staphylococcus aureus* in Nederland. 2002. Available at http://www.nvmm.nl.

Netherlands Society for Medical Microbiology (NVMM). NVMM-richtlijn voor screening en confirmatie van extended-spectrum beta-lactamases (ESBLs) in Enterobacteriaceae. 2008. Available at http://www.nvmm.nl.

Netherlands Society for Medical Microbiology (NVMM). Protocol voor de ontwikkeling, autorisatie en revisie van beroepsgebonden richtlijnen van de Nederlandse Vereniging voor Medische Microbiologie. 2011. Available at http://www.nvmm.nl.

Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 2005;18:657-86.

Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev 2007; 20:440-58.

Shahangian S, Snyder SR. Laboratory medicine quality indicators. A review of the literature. Am J Clin Pathol 2009;131:418-31.

Steindel SJ, Howanitz PJ. Changes in emergency department turnaround time performance from 1990 to 1993. A comparison of two College of American Pathologists Q-probes studies. Arch Pathol Lab Med 1997;121:1031-41.

Steindel SJ, Novis DA. Using outlier events to monitor test turnaround time. A College of American Pathologists Q-probes study in 496 laboratories. Arch Pathol Lab med 1999;123:607-14.

SWAB. Nethmap 2010. Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. 2010. Available at http://www/swab.nl.

Valenstein PN, Emancipator K. Sensitivity, specificity, and reproducibility of four measures of laboratory turnaround time. Am J Clin Pathol 1989;91:452-7.

VANTURES. MARAN 2008. Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2008. Available at http://ww.cvi.wur.nl.

Wassenberg MWM, Kluytmans JAJW, Box ATA, Bosboom RW, Buiting AGM, Elzakker EPM, Melchers WJG, van Rijen MML, Thijsen SFT, Troelstra A, Vandenbroucke-Grauls CMJE, Visser CE, Voss A, Wolffs PFG, Wulf MWH, van Zwet AA, de Wit GA, Bonten MJM. Rapid screening of methicillin-resistant *Staphylococcus aureus* using PCR and chromogenic agar: a prospective study to evaluate costs and effects. CMI 2010;16:1754-61.

Chapter 2 – *Staphylococcus aureus*

2.1 Methicillin resistance

Acton DS, Tempelmans Plat-Sinnige MJ, van Wamel W, de Groot N, van Belkum A. Intestinal carriage of *Staphylococcus aureus*: how does its frequency compare with that of nasal carriage and what is its clinical impact? Eur J Clin Microbiol Infect Dis. 2009;28:115-27.

Aldeyab MA, Kearney MP, Hughes CM, Scott MG, Tunney MM, Gilpin DF, Devine MJ, Watson JD, Gardiner A, Funston C, Savage K, McElnay JC. Can the use of a rapid polymerase chain screening method decrease the incidence of nosocomial meticillin-resistant *Staphylococcus aureus*? J Hosp Infect 2009;71:22-8.

Bannerman TL. *Staphylococcus*, *Micrococcus* and other catalase-positive cocci that grow aerobically. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, editors. Manual of clinical microbiology, 8th edition. ASM Press, Washington D.C., 2003.

Böcher S, Smyth R, Kahlmeter G, Kerremans J, Vos MC, Skov R. Evaluation of four selective agars and two enrichment broths in screening for methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 2008;46:3136-8.

Böcher S, Middendorf B, Westh H, Mellmann A, Becker K, Skov R, Friedrich AW. Semi-selective broth improves screening for methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 2010;65:717-20.

Bruins MJ, Juffer P, Wolfhagen MJHM, Ruijs GJHM. Salt tolerance of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus*. J Clin Microbiol 2007;45:682-3.

Chambers HF, Archer G, Matsuhashi M. Low-level methicillin resistance in strains of *Staphylococcus aureus*. Antimicrob Agents Chemother 1989;33:424-8.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement. 2007. M100-S17.

Coello R, Kiménez J, García M, Arroyo P, Minguez D, Fernández C, Cruzet F, Gaspar C. Prospective study of infection, colonization and carriage of methicillin-resistant *Staphylococcus aureus* in an outbreak affecting 990 patients.

De Silva S, Wood G, Quek T, Parrott C, Bennett C. Comparison of flocked and rayon swabs for detection of nasal carriage of *Staphylococcus aureus* among pathology staff members. J Clin Microbiol 2010;48:2963-4.

Diederen BMW. Comparison of the Cepheid Xpert MRSA assay with culture in a low prevalence setting in The Netherlands. J Infect 2010;61:509-10.

Dutch Workingparty on Infection Prevention. MRSA hospital. 2007. Available at: http://www.wip.nl.

EUCAST. Breakpoint tables for interpretation of MICs and zone diameters. Version 2.0, 2012. Available at http://www.eucast.org.

Grmek-Kosnik I, Ihan A, Dermota U, Rems M, Kosnik M, Jorn Kolmos H. Evaluation of separate vs pooled swab cultures, different media, broth enrichment and anatomical sites of screening for the detection of methicillin-resistant *Staphylococcus aureus* from clinical specimens. J Hosp Infect 2005;155-61.

van Griethuysen A, Pouw M, van Leeuwen N, Heck M, Willemse P, Buiting A, Kluytmans J. J Clin Microbiol 1999;37:2789-92.

Harbarth S, Masuet-Aumatell C, Schrenzel J, Francois P, Akakpo C, Renzi G, Pugin J, Ricou B, Pittet D. Evaluation of rapid screening and pre-emptive contact isolation for detecting and controlling methicillin-resistant *Staphylococcus aureus* in critical care: an interventional cohort study. Crit Care 2006;10:R25.

Hartman BJ, Tomasz A. Low-affinity penicillin-binding protein associated with beta-lactam resistance in *Staphylococcus aureus*. J Bacteriol 1984;158:513-6.

van Horn KG, Audette CD, Sebeck D, Tucker KA. Comparison of the Copan ESwab system with two Amies agar swab transport systems for maintenance of microorganism viability. J Clin Microbiol 2008;46:1655-8.

Jevons MP. “Celbenin”-resistant staphylococci. Br Med J 1961;1:124-5.

Jeyaratnam D, Whitty CJ, Phillips K, Liu D, Orezzi C, Ajoku U, French GL. Impact of rapid screening tests on acquisition of meticillin resistant *Staphylococcus aureus*: cluster randomised crossover trial. Br Med J 2008;336:927-34. (A)

Jeyaratnam D, Gottlieb A, Ajoku U, French GL. Validation of the IDI-MRSA system for use on pooled nose, axilla, and groin swabs and single swabs from other screening sites. Diagn Microbiol Infect Dis 2008;61:1-5. (B)

Jones EM, Bowker KE, Cooke R, Marshall RJ, Reeves DS, MacGowan AP. Salt tolerance of EMRSA-16 and its effect on the sensitivity of screening cultures. J Hosp Infect 1997;35:59-62.

Kelley PG, Grabsch EA, Howden BP, Gao W, Grayson ML. Comparison of the Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) assay, BD GeneOhm MRSA assay, and culture for detection of nasal and cutaneous groin colonization by MRSA. J Clin Microbiol 2009;47:3769-72.

Kerremans JJ, Maaskant J, Verbrugh HA, van Leeuwen WB, Vos MC. Detection of methicillin-resistant *Staphylocoocus aureus* in a low-prevalence setting by polymerase chain reaction with a selective enrichment broth. Diagn Microbiol Infect Dis 2008;61:396-401.

Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 1997;10:505-20.

Larsson AK, Gustafsson E, Nilsson AC, Odenholt I, Ringberg H, Melander E. Duration of methicillin-resistant *Staphylococcus aureus* colonization after diagnosis: a four-year experience from southern Sweden. Scand J Infect Dis 2011;43:456-62.

Lauderdale TL, Wang JT, Lee WS, Huang JH, McDonald LC, Huang IW, Chang SC. Carriage rates of methicillin-resistant *Staphylococcus aureus* (MRSA) depend on anatomic location, the number of sites cultured, culture methods, and the distribution of clonotypes. Eur J Clin Microbiol Infect Dis 2010;29:1553-9.

Lautenbach E, Nachamkin I, Hu B, Fishman NO, Tolomeo P, Prasad P, Bilker WB, Zaoutis TE. Surveillance cultures for detection of methicillin-resistant *Staphylococcus aureus*: diagnostic yield of anatomic sites and comparison of provider- and patient-collected samples. Infect Control Hosp Epidemiol 2009;30:380-2.

Leclercq R, Cantón R, Brown DFJ, Giske CG, Heisig P, MacGowan AP, Mouton JW, Nordmann P, Rodloff AC, Rossolini GM, Soussy CJ, Steinbakk M, Winstanley TG, Kahlmeter G. EUCAST expert rules in antimicrobial susceptibility testing. CMI 2011;doi 10.1111/j.1469-0691.2011.03703.x.

de Lencastre H, Sá Figueiredo AM, Urban C, Rahal J, Tomasz A. Multiple mechanisms of methicillin resistance and improved methods for detection in clinical isolates of *Staphylococcus aureus*. Antimicrob Agents Chemother 1991;35:632-9.

de Lencastre H, Tomasz A. Reassessment of the number of auxiliary genes essential for expression of high-level methicillin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 1994;38:2590-8.

Lindqvist M, Isaksson B, Grub C, Jonassen TØ, Hällgren A. Detection and characterisation of SCC*mec* remnants in multiresistant methicillin-susceptible *Staphylococcus aureus* causing a clinal outbreak in a Swedish county. Eur J Clin Microbiol Infect Dis 2012;31:141-7.

Luteijn JM, Hubben GAA, 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;17:146-54.

Malhotra-Kumar S, Haccuria K, Michiels M, Ieven M, Poyart C, Hryniewicz W, Goossens H, on behalf of the MOSA WP2 Study Team. Current trends in rapid diagnostics for methicillin-resistant *Staphylococcus aureus* and glycopeptide-resistant *Enterococcus* species. J Clin Microbiol 2008;46:1577-87.

Malhotra-Kumar S, Abrahantes JC, Sabiiti W, Lammens C, Vercauteren G, Ieven M, Molenberghs G, Aerts M, Goossens H, on behalf of the MOSAR WP2 Study Team. Evaluation of chromogenic media for detection of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 2010;48:1040-6.

Marschall J, Mühlemann K. Duration of methicillin-resistant *Staphylococcus aureus* carriage, according to risk factors for acquisition. Infect Control Hosp Epidemiol 2006;27:1206-12.

Matheson A, Christie P, Stari T, Kavanagh K, Gould IM, Masterton R, Reilly JS. Nasal swab screening for methicillin-resistant *Staphylococcus aureus* – How well does it perform ? A cross-sectional study. Infect Control Hosp Epidemiol 2012 ;33 :803-8.

Matsuhashi M, Song MD, Ishino F, Wachi M, Doi M, Inoue M, Ubukata K, Yamashita N, Konno M. Molecular cloning of the gene of a penicillin-binding protein supposed to cause high resistance to beta-lactam antibiotics in *Staphylococcus aureus*. J Bacteriol 1986;167:975-80.

McAllister SK, Albrecht VS, Fosheim GE, Lowery HK, Peters PJ, Gorwitz R, Guest JL, Hageman J, Mindley R, McDougal LK, Rimland D, Limbago B. Evaluation of the impact of direct plating, broth enrichment, and specimen source on recovery and diversity of methicillin-resistant *Staphylococcus aureus* isolates among HIV-infected outpatients. J Clin Microbiol 2011;49:4126-30.

McDougal LK, Thornsberry C. The role of beta-lactamase in staphylococcal resistance to penicillinase-resistant penicillins and cephalosporins. J Clin Microbiol 1986;23:832-9.

Mertz D, Frei R, Jaussi B, Tietz A, Stebler C, Flückiger U, Widmer AF. Throat swabs are necessary to reliably detect carriers of *Staphylococcus aureus*. Clin Infect Dis 2007;45:475-7.

Mollema FPN, Severin JA, Nouwen JL, Ott A, Verbrugh HA, Vos MC. Succesful treatment for carriage of methicillin-resistant *Staphylocccus aureus* and importance of follow-up. Antimicrob Agents Chemother 2010;54:4020-5.

Montanari MP, Tonin E, Biavasco F, Varaldo PE. Further characterization of borderline methicillin-resistant *Staphylococcus aureus* and analysis of penicillin-binding proteins. Antimicrob Agents Chemother 1990;34:911-3.

Perry JD, Freydière. The application of chromogenic media in clinical microbiology. J Appl Microbiol 2007;103:2046-55.

Reynolds PE, Brown DF. Penicillin-binding proteins of beta-lactam-resistant strains of *Staphylococcus aureus*. Effect of growth conditions. FEBS Letters 1985;192:28-32.

Riewerts Eriksen NH, Espersen F, Thamdrup Rosdahl V, Jensen K. Evaluation of methods for the detection of nasal carriage of Staphylococcus aureus. APMIS 1994;102:407-12.

Robicsek A, Beaumont JL, Peterson LR. Duration of colonization with methicillin-resistant *Staphylococcus aureus*. Clin Infet Dis 2009;48:910-3.

Roisin S, Laurent C, Nonhoff C, Deplano A, Hallin M, Byl B, Struelens MJ, Denis O. Positive predictive value of the Xpert MRSA assay diagnostic for universal patient screening at hospital admission: influence of the local ecology. Eur J Clin Microbiol Infect Dis 2012;31:873-80.

Rossney AS, Herra CM, Brennan GI, Morgan PM, O'Connell B. Evaluation of the Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) assay using the GeneXpert real-time PCR platform for rapid detection of MRSA from screening specimens. J Clin Microbiol 2008;46:3285-90.

Saegeman V, Flamaing J, Muller J, Peetermans WE, Stuyck J, Verhaegen J. Clinical evaluation of the Copan ESwab for methicillin-resistant *Staphylococcus aureus* detection and culture of wounds. Eur J Clin Microbiol Infect Dis 2011;30:943-9.

Sakoulas G, Gold HW, Venkataraman L, Degirolami PC, Eliopoulos GM, Qian Q. Methicillin-resistant *Staphylococcus aureus*: comparison of susceptibility testing methods and analysis of *mec*A-positive susceptibile strains. J Clin Microbiol 2001;39:3946-51.

Sanford MD, Widmer AF, Bale MJ, Jones RN, Wenzel RP. Efficient detection and long-term persistence of the carriage of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 1994;19:1123-8.

Scanvic A, Denic L, Gaillon S, Giry P, Andremont A, Lucet JC. Duration of colonization by methicillin-resistant *Staphylococcus aureus* after hospital discharge and risk factors for prolonged carriage. Clin Infect Dis 2001;32:1393-8.

Senn L, Basset P, Nahimana I, Zanetti G, Blanc DS. Which anatomical sites should be sampled for screening of methicillin-resistant *Staphylococcus aureus* by culture or by rapid PCR test? Clin Microbiol Infect 2012;18:E31-3.

Skov R, Smyth R, Clausen M, Larsen AR, Frimodt-Møller N, Olsson-Liljequist B, Kahlmeter G. Evaluation of a cefoxitin 30 ug disc on Iso-Sensitest agar for detection of methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 2003;52:204-7.

Skov R, Smyth R, Yusof A, Karlsson A, Mills K, Frimodt-Møller N, Kahlmeter G. Effects of temperature on the detection of methicillin resistance in *Staphylococcus aureus* using cefoxitin disc diffusion testing with Iso-Sensitest agar. J Antimicrob Chemother 2009;63:699-703.

Smismans A, Verhaegen J, Schuermans A, Frans J. Evaluation of the Copan ESwab transport system for the detection of methicillin-resistant *Staphylocoocus aureus*: a laboratory and clinical study. Diagn Microbiol Infect Dis 2009;65:108-11.

Smyth RW, Kahlmeter G, Olsson Liljequist B, Hoffman B. Methods for identifying methicillin resistance in *Staphylococcus aureus*. J Hosp Infect 2001;48:103-7.

Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale C, Skov R, Larsen AR. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mec*A or the new *mec*A homologue *mec*A_{LGA251} Clin Microbiol Infect 2012;18:395-400.

Svent-Kucina N, Pirs M, Mueller-Premru M, Cvitkovic-Spik V, Kofol R, Seme K. One-year experience with modified BD GeneOhm MRSA assay for detection of methicillin-resistant *Staphylococcus aureus* from pooled nasal, skin, and throat samples. Diagn Microbiol Infect Dis 2009;63:132-9.

Stichting Werkgroep Antibioticabeleid (SWAB). Optimaliseren van het antibioticabeleid in Nederland XII. Herziening SWAB richtlijn Behandeling MRSA dragers. 2012.

Uçkay I, Sax H, Iten A, Camus V, Renzi G, Schrenzel J, Perrier A, Pittet D. Effect of screening for methicillin-resistant *Staphylococcus aureus* carriage by polymerase chain reaction on the duration of unnecessary preemptive contact isolation. Infect Control Hosp Epidemiol 2008;29:1077-9.

Vandenbergh MFQ, Verbrugh HA. Carriage of *Staphylococcus aureus*: epidemiology and clinical relevance. J Lab Clin Med 1999;133:525-34.

Vandenbroucke-Grauls CMJE. Management of methicillin-resistant *Staphylococcus aureus* in The Netherlands. Rev Med Microbiol 1998;9:109-16.

Verhoeven P, Grattard F, Carricajo A, Pozzetto B, Berthelot P. Better detection of *Staphylococcus aureus* nasal carriage by use of nylon flocked swabs. J Clin Microbiol 2010;48:4242-4.

Verkade E, Ferket M, Kluytmans J. Clinical evaluation of Oxoid *Brilliance* MRSA Agar in comparison with bioMérieux MRSA ID medium for detection of livestock-associated meticillin-resistant *Staphylococcus aureus*. J Med Microbiol 2011;60:905-8.

Wassenberg MWM, Kluytmans JAJW, Box ATA, Bosboom RW, Buiting AGM, van Elzakker EPM, Melchers WJG, van Rijen MML, Thijsen SFT, Troelstra A, Vandenbroucke-Grauls CMJE, Visser CE, Voss A, Wolffs PFG, Wulf MWH, van Zwet AA, de Wit GA, Bonten MJM. Rapid screening of methicillin-resistant *Staphylococcus aureus* using PCR and chromogenic agar: a prospective study to evaluate costs and effects. Clin Microbiol Infection 2010;16:1754-61.

Wassenberg MWM, Kluytmans JAJW, Bosboom RW, Buiting AGM, van Elzakker EPM, Melchers WJG, Thijsen SFT, Troelstra A, Vandenbrouke-Grauls CMJE, Visser CE, Voss A, Wolffs PFG, Wulf MWH, van Zwet AA, de Wit GA, Bonten MM. Rapid diagnostic testing of methicillin-resistant *Staphylococcus aureus* carriage at different anatomical sites: costs and benefits of less extensive screening regimens. Clin Microbiol Infect 2011;17:1704-10.

Wertheim FL, Verveer J, Boelens HAM, van Belkum A, Verbrugh HA, Vos MC. Effect of mupirocin treatment on nasal, pharyngeal, and perineal carriage of *Staphylococcus aureus* in healthy adults.

Wolk DM, Picton E, Johnson D, Davis T, Pancholi P, Ginocchio CC, Finegold S, Welch DF, de Boer M, Fuller D, Solomon MC, Rogers B, Mehta MS, Peterson LR. Multicenter evaluation of the Cepheid Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) test as a rapid screening method for detection of MRSA in nares. J Clin Microbiol 2009;47:758-64.

Chapter 5 – Enterobacteriaceae

5.1 Extended-spectrum beta-lactamases

Bedenic B, Vranes J, Mihaljevic LJ, Tonkic M, Sviben M, Plecko V, Kalenic S. Sensitivity and specificity of various beta-lactam antibiotics and phenotypical methods for detection of TEM, SHV and CTX-M extended-spectrum beta-lactamases. J Chemother 2007;19:127-39.

Behravesh CB, Lynch M, Schlundt J. Salmonellosis. Control of Communicable Diseases Manual 2008, Heymann DL, 19th edition.

Biedenbach DJ, Toleman M, Walsh TR, Jones RN. Analysis of *Salmonella* spp. with resistance to extended-spectrum cephalosporins and fluoroquinolones isolated in North America and Latin America: report from the SENTRY Antimicrobial Surveillance Program (1997-2004). Diagn Microbiol Infect Dis 2006;54:13-21.

Bradford PA. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 2001;14:933-51.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement. 2011. M100-S21.

Cohen Stuart J, Dierikx C, Al Naiemi N, Karcmarek A, Van Hoek AH, Vos P, Fluit AC, Scharringa J, Duim B, Mevius D, Leverstein-van Hall MA. Rapid detection of TEM, SHV and CTX-M extended-spectrum beta-lactamases in Enterobacteriaceae using ligation-mediated amplification with microarray analysis. J Antimicrob Chemoth 2010;65:1377-81.

Cohen Stuart J, Diederen B, al Naiemi N, Fluit A, Arents N, Thijsen S, Vlaminckx B, Mouton JW, Leverstein-van Hall M. Method for phenotypic detection of extended-spectrum beta-lactamases in *Enterobacter* species in the routine clinical setting. J Clin Microbiol 2011;49:2711-13.

Drieux L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detection of extended-spectrum beta-lactamase production in *Enterobacteriaceae*: review and bench guide. Clin Microbiol Infect 2008;14 (Suppl.1):90-103.

Endimiani A, Hujer AM, Hujer KM, Gatta JA, Schriver AC, Jacobs MR, Rice LB, Bonomo RA. Evaluation of a commercial microarray system for detection of SHV-, TEM-, CTX-M, and KPC-type beta-lactamase genes in Gram-negative isolates. J Clin Microbiol 2010;48:2618-22.

EUCAST. Breakpoint tables for interpretation of MICs and zone diameters. Version 2.0, 2012. Available at http://www.eucast.org

Hart CA, Gibson MF. Comparative epidemiology of gentamicin-resistant enterobacteria: persistence of carriage and infection. J Clin Pathol 1982;35:452-7.

Hirakata Y, Matsuda J, Miyazaki Y, Kamihira S, Kawakami S, Miyazawa Y, Ono Y, Nakazaki N, Hirata Y, Inoue M, Turnidge JD, Bell JM, Jones RN, Kohno S, the SENTRY Asia-Pacific participants. Regional variation in the prevalence of extended-spectrum beta-lactamase-producing clinical isolates in the Asia-Pacific region (SENTRY 1998-2002). Diagn Microbiol Infect Dis 2005;52:323-9.

Health Protection Agency (HPA). Laboratory detection and reporting of bacteria with extended spectrum beta-lactamases. 2008. National Standard Method QSOP 51 Issue 2.2. Available at http://www.hpa-standardmethods.org.uk

Hope R, Potz NAC, Warner M, Fagan EJ, Arnold E, Livermore DM. Efficacy of practiced screening methods for detection of cephalosporin-resistant Enterobacteriaceae. J Antimicrob Chemother 2007;59:110-3.

Huang TD, Bogaerts P, Berhin C, Guisset A, Glupczynski Y. Evaluation of Brilliance ESBL agar, a novel chromogenic medium for detection of extended-spectrum-beta-lactamase-producing Enterobacteriaceae. J Clin Microbiol 2010;48:2091-6.

Jacoby GA. AmpC beta-lactamases. Clin Microbiol Rev 2009;22:161-82.

Jeong SH, Song W, Kim JS, Kim HS, Lee KM. Broth microdilution method to detect extended-spectrum beta-lactamases and AmpC beta-lactamases in Eenterobacteriaceae isolates by use of clavulanic acid and boronic acid as inhibitors. J Clin Microbiol 2009;47:3409-12

Kim S, Kim J, Kang Y, Park Y, Lee B. Occurrence of extended-spectrum beta-lactamases in members of the genus *Shigella* in the Republic of Korea. J Clin Microbiol 2004;42:5264-9.

Lahey Clinic. Beta-lactamase classification and amino acid sequences for TEM, SHV and OXA extended-spectrum and inhibitor resistant enzymes. 2011. Available at: www.lahey.org/studies.

Lautenbach E, Harris AD, Perencevich EN, Nachamkin I, Tolomea P, Metlay JP. Test characteristics of perirectal and rectal swab compared to stool sample for detection of fluoroquinolone-resistant *Escherichia coli* in the gastrointestinal tract. Antimicrob Agents Chemother 2005;49:798-800.

Leclercq R, Cantón R, Brown DFJ, Giske CG, Heisig P, MacGowan AP, Mouton JW, Nordmann P, Rodloff AC, Rossolini GM, Soussy CJ, Steinbakk M, Winstanley TG, Kahlmeter G. EUCAST expert rules in antimicrobial susceptibility testing. CMI 2011;doi 10.1111/j.1469-0691.2011.03703.x.

Leverstein-van Hall MA, Fluit AC, Paauw A, Box ATA, Brisse S, Verhoef J. Evaluation of the Etest ESBL and the BD Phoenix, VITEK 1, and VITEK 2 automated instruments for detection of extended-spectrum beta-lactamases in multiresistant *Escherichia coli* and *Klebsiella* spp. J Clin Microbiol 2002;40:3703-11.

Livermore DM. Beta-lactamases in laboratory and clinical resistance. Clin Microbiol Rev 1995;8:557-84.

Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, Ayala J, Coque TM, Kern-Zdanowicz I, Luzzaro F, Poirel L, Woodford N. CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother 2007;59:165-74.

March A, Aschbacher R, Dhanji H, Livermore DM, Böttcher A, Sleghel F, Maggi S, Noale M, Larcher C, Woodford N. Colonization of residents and staff of a long-term-care facility and adjacent acute-care hospital geriatric unit by multiresistant bacteria. Clin Microbiol Infect 2010;16:934-44.

Moore G, Griffith C. Problems associated with traditional hygiene swabbing: the need for in-house standardization. J Appl Microbiol 2007;103:1090-103.

Munier GK, Johnson CL, Snyder JW, Moland ES, Hanson ND, Thomson KS. Positive extended-spectrum-beta-lactamase (ESBL) screening results may be due to AmpC beta-lactamases more often than to ESBLs. J Clin Microbiol 2010;48:673-4.

Murk JLAN, Heddema ER, Hess DLJ, Bogaards JA, Vandenbroucke-Grauls CMJE, Debets-Ossenkop YJ. Enrichment broth improved detection of extended-spectrum-beta-lactamase-producing bacteria in throat and rectal surveillance cultures of samples from patients in intensive care units. J Clin Microbiol 2009;47:1885-7.

M'Zali FH, Chanawong A, Kerr KG, Birkenhead D, Hawkey PM. Detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae: comparison of the MAST DD test, the double disc and the Etest ESBL. J Antimicrob Chemother 2000;45:881-5.

Naas T, Poirel L, Nordmann P. Minor extended-spectrum beta-lactamases. Clin Microbiol Infect 2008 14 (Suppl 1):42-52.

Naas T, Cuzon G, Truong H, Bernabeu S, Nordmann P. Evaluation of a DNA microarray, the Check-Points ESBL/KPC array, for rapid detection of TEM, SHV, and CTX-M extended-spectrum beta-lactamases and KPC carbapenemases. Antimicrob Agents Chemother 2010;54:3086-92.

al Naiemi al N, Bart A, de Jong MD, Vandenbroucke-Grauls CM, Rietra PJ, Debets-Ossenkopp YJ, Wever PC, Spanjaard L, Bos AJ, Duim B. Widely distributed and predominant CTX-M extended-spectrum beta-lactamases in Amsterdam, The Netherlands. J Clin Microbiol 2006; 44:3012-4.

Navarro F, Perez-Trallero E, Marimon JM, Aliaga R, Gomariz M, Mirelis B. CMY-2-producing *Salmonella enterica*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis* and *Escherichia coli* strains isolated in Spain (October 1999-December 2000). J Antimicrob Chemother 2001;48:383-9.

Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect Dis 2009;9:228-36.

Overdevest ITMA, Willemsen I, Elberts S, Verhulst C, Kluytmans JAJW. Laboratory detection of extended-spectrum-beta-lactamase-producing *Enterobacteriaceae*: Evaluation of two screening agar plates and two confirmation techniques. J Clin Microbiol 2011;49:519-22.

Paniagua R, Valverde A, Coque TM, Baquero F, Cantón R. Assessment of prevalence and changing epidemiology of extended-spectrum beta-lactamase-producing Enterobacteriaceae fecal carriers using a chromogenic medium. Diagn Microbiol Infect Dis 2010;67:376-9.

Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 2005;18:657-86.

Platteel TN, Cohen Stuart JW, Voets GM, Scharringa J, van de Sande N, Fluit AC, Leverstein-Van Hall MA; on behalf of the ESBL national surveillance working group. Evaluation of a commercial microarray as a confirmation test for the presence of extended-spectrum beta-lactamases in isolates from the routine clinical setting. Clin Microbiol Infect 2011. doi:10.1111/j.1469-0691.2011.03567.x

Sanders, CC, Barry AL, Washington JA, Shubert C, Moland ES, Traczewski MM, Knapp C, Mulder R. Detection of extended-spectrum-beta-lactamase-producing members of the family *Enterobacteriaceae* with the Vitek ESBL test. J Clin Microbiol 1996;34:2997-3001.

Sirot D, Recule C, Chaibi EB, Bret L, Croize J, Chanal-Claris C, Labia R, Sirot J. A complex mutant of TEM-1 beta-lactamase with mutations encountered in both IRT-4 and extended-spectrum TEM-15, produced by an *Escherichia coli* clinical isolate. Antimicrob Agents Chemother 1997;41:1322-5.

Spanu T, Sanguinetti M, Tumbarello M, D'Inzeo T, Fiori B, Posteraro B, Santangelo R, Cauda R, Fadda G. Evaluation of the new VITEK 2 extended-spectrum beta-lactamase (ESBL) test for rapid detection of ESBL production in Enterobacteriaceae isolates. J Clin Microbiol 2006;44:3257-62.

Stürenburg E, Sobottka I, Noor D, Laufs R, Mack D. Evaluation of a new cefepime-clavulanate ESBL Etest to detect extended-spectrum beta-lactamases in an Enterobacteriaceae strain collection. J Antimicrob Chemother 2004;54:134-8.

Thomson KS, Cornish NE, Hong SG, Hemrick K, Herdt C, Moland ES. Comparison of Phoenix and VITEK 2 extended-spectrum-beta-lactamase detection tests for analysis of *Escherichia coli* and *Klebsiella* isolates with well-characterized beta-lactamases. J Clin Microbiol 2007;45:2380-4.

Towne TG, Lewis JS II2nd, Herrera M, Wickes B, Jorgenson JH. Detection of SHV-type extended-spectrum beta-lactamase in *Enterobacter* isolates. J Clin Microbiol 2010;48:298-9.

Tzelepi E, Giakkoupi P, Sofianou D, Loukova V, Kemeroglou A, Tsakris A. Detection of extended-spectrum beta-lactamases in clinical isolates of *Enterobacter cloacae* and *Enterobacter aerogenes*. J Clin Microbiol 2000;38:542-6.

Voets GM, Fluit AC, Scharringa J, Cohen Stuart J, Leverstein-van Hall MA. A set of multiplex PCRs for genotypic detection of extended-spectrum beta-lactamases, carbapenemases, plasmid-mediated AmpC beta-lactamases and OXA-beta-lactamases. Int J Antimicrob Agents 2011;37:356-9.

Wiener-Well Y, Rudensky B, Yinnon AM, Kopuit P, Schlesinger Y, Broide E, Lachish T, Raveh D. Carriage rate of carbapenem-resistant *Klebsiella pneumoniae* in hospitalised patients during a national outbreak. J Hosp Infect 2010;74:344-9.

Willemsen I, Overdevest I, al Naiemi N, Rijnsburger M, Savelkoul P, Vandenbroucke-Grauls C, Kluytmans J; on behalf of the TRIANGLe study group. A new diagnostic microarray (Check-KPC ESBL) for detection and identification of extended-spectrum beta-lactamases in highly resistant Enterobacteriaceae. J Clin Microbiol 2011;49:2985-7.

Woodford N, Reddy S, Fagan EJ, Hill RL, Hopkins KL, Kaufmann ME, Kistler J, Palepou MF, Pike R, Ward MW, Cheesbrough J, Livermore DM. Wide geographic spread of diverse acquired AmpC beta-lactamases among *Escherichia coli* and *Klebsiella* spp. in the UK and Ireland. J Antimicrob Chemother 2007;59:102-5.

Wu TL, Siu LL, Siu LH, Lauderdale TL, Lin FM, Leu HS, Lin TY, Ho M. Outer membrane protein change combined with co-existing TEM-1 and SHV-1 beta-lactamases lead to false identification of ESBL-producing *Klebsiella pneumoniae*. J Antimicrob Chemother 2001;47:755-61.

Yagci D, Yoruk F, Azap A, Memikoglu O. Prevalence and risk factors for selection of quinolone-resistant *Escherichia coli* strains in fecal flora of patients receiving quinolone therapy. Antimicrob Agents Chemother 2009;53:1287-9.

5.2 Carbapenemases

Adler A, Navon-Venezia S, Moran-Gilad J, Marcos E, Schwartz D, Carmeli Y. Laboratory and clinical evaluation of screening agar plates for detection of carbapenem-resistant Enterobacteriaceae from surveillance rectal swabs. J Clin Microbiol 2011;49:2239-42.

Bedenic B, Vranes J, Mihaljevic LJ, Tonkic M, Sviben M, Plecko V, Kalenic S. Sensitivity and specificity of various beta-lactam antibiotics and phenotypical methods for detection of TEM, SHV and CTX-M extended-spectrum beta-lactamases. J Chemother 2007;19:127-39.

Behravesh CB, Lynch M, Schlundt J. Salmonellosis. Control of Communicable Diseases Manual 2008, Heymann DL, 19th edition.

Bratu S, Landman D, Haag R, Recco R, Eramo A, Alam M, Quale J. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: a new threat to our antibiotic armamentarium. Arch Intern Med 2005;165:1430-5.

Carrër A, Forineau N, Nordmann P. Use of ChromID extended-spectrum beta-lactamase medium for detecting carbapenemase-producing *Enterobacteriaceae*. J Clin Microbiol 2010;48:1913-4.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement. 2011. M100-S21.

Cohen Stuart J, Dierikx C, Al Naiemi N, Karcmarek A, Van Hoek AH, Vos P, Fluit AC, Scharringa J, Duim B, Mevius D, Leverstein-van Hall MA. Rapid detection of TEM, SHV and CTX-M extended-spectrum betal-lactamases in Enterobacteriaceae using ligation-mediated amplification with microarray analysis. J Antimicrob Chemoth 2010;65:1377-81.

Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. J Antimicrob Chemother 2010;65:490-5.

EUCAST. Breakpoint tables for interpretation of MICs and zone diameters. Version 2.0, 2012. Available at: http://www.eucast.org

Falcone M, Mezzatesta ML, Perilli M, Forcella C, Giordano A, Cafiso V, Amicosante G, Stefani S, Venditti M. Infections with VIM-1 metallo-beta-lactamase-producing *Enterobacter cloacae* and their correlation with clinical outcome. J Clin Microbiol 2009;47:3514-9.

Giske CG, Gezelius L, Samuelsen Ø, Warner M, Sundsfjord A, Woodford N. A sensitive and specific phenotypic assay for detection of metallo-beta-lactamases and KPC in *Klebsiella pneumoniae* with the use of meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin. Clin Microbiol Infect 2011;17:552-6.

Hart CA, Gibson MF. Comparative epidemiology of gentamicin-resistant enterobacteria: persistence of carriage and infection. J Clin Pathol 1982;35:452-7.

Lautenbach E, Harris AD, Perencevich EN, Nachamkin I, Tolomea P, Metlay JP. Test characteristics of perirectal and rectal swab compared to stool sample for detection of fluoroquinolone-resistant *Escherichia coli* in the gastrointestinal tract. Antimicrob Agents Chemother 2005;49:798-800.

Leclercq R, Cantón R, Brown DFJ, Giske CG, Heisig P, MacGowan AP, Mouton JW, Nordmann P, Rodloff AC, Rossolini GM, Soussy CJ, Steinbakk M, Winstanley TG, Kahlmeter G. EUCAST expert rules in antimicrobial susceptibility testing. CMI 2011;doi 10.1111/j.1469-0691.2011.03703.x.

Miriagou V, Cornaglia G, Edelstein M, Galani I, Giske CG, Gniadkowski M, Malamou-Lada E, Martinez-Martinez L, Navarro F, Nordmann P, Peixe L, Pournaras S, Rossolini GM, Tsakris A, Vatopoulos A, Cantón R. Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. Clin Microbiol Infect 2010;16:112-22.

Moore G, Griffith C. Problems associated with traditional hygiene swabbing: the need for in-house standardization. J Appl Microbiol 2007;103:1090-103.

Moran Gilad J, Carmeli Y, Schwartz D, Navon-Venezia S. Laboratory evaluation of the CHROMagar KPC medium for identification of carbapenem-nonsusceptible Enterobacteriaceae. Diagn Microbiol Infect Dis 2011;70:565-7.

Naas T, Cuzon G, Bogaerts P, Glupczynski Y, Nordmann P. Evaluation of a DNA microarray (Check-MDR CT102) for rapid detection of TEM, SHV, and CTX-M extended-spectrum beta-lactamases and of KPC, OCA-48, VIM, IMP and NDM-1 carbapenemases. J Clin Microbiol 2011;49:1608-13.

Nordman P, Poirel L, Carrër A, Toleman MA, Walsh TR. How to detect NDM-1 producers. J Clin Micorbiol 2011;49:718-21.

Paniagua R, Valverde A, Coque TM, Baquero F, Cantón R. Assessment of prevalence and changing epidemiology of extended-spectrum beta-lactamase-producing Enterobacteriaceae fecal carriers using a chromogenic medium. Diagn Microbiol Infect Dis 2010;67:376-9.

Panagea T, Galani I, Souli M, Adamou P, Antoniadou A, Giamarellou H. Evaluation of CHROMagar™ KPC for the detection of carbapenemase-producing Enterobacteriaceae in rectal surveillance cultures. Int J Antimicrob Agents 2011;37:124-8.

Pasteran F, Mendez T, Guerriero L, Rapoport M, Corso A. Sensitive screening tests for suspected class A carbapenemase production in species of Enterobacteriaceae. J Clin Microbiol 2009;47:1631-9.

Pasteran F, Mendez T, Rapoport M, Guerriero L, Corso A. Controlling false-positive results obtained with the Hodge and Masuda assays for detection of class A carbapenemase in species of Enterobacteriaceae by incorporating boronic acid. J Clin Microbiol 2010;48:1323-32.

Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 2005;18:657-86.

Poirel L, Bernabeu S, Fontineau N, Podglajen I, Lawrence C, Nordmann P. Emergence of OXA-48-producing *Escherichia coli* clone ST38 in France. Antimicrob Agents Chemother 2011;55:4937-8.

Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev 2007; 20:440-58.

Samra Z, Bahar J, Madar-Shapiro L, Aziz N, Israel S, Bishara J. Evaluation of CHROM agar KPC for rapid detection of carbapenem-resistant Enterobacteriaceae. J Clin Microbiol 2008;46:3110-1.

Souli M, Galani I, Antoniadou A, Papadomichelakis E, Poulakou G, Panagea T, Vourli S, Zerva L, Armaganidis A, Kanellakopoulou K, Giamarellou H. An outbreak of infection due to beta-lactamase *Klebsiella pneumoniae* carbapenemase 2-producing *K. pneumoniae* in a Greek university hospital: molecular characterization, epidemiology, and outcomes. Clin Infect Dis 2010;50:364-73.

Tenover FC, Kalsi RK, Williams PP, Carey RB, Stocker S, Lonsway D, Rasheed JK, Biddle JW, McGowan JE, Hanna B. Carbapenem resistance in *Klebsiella pneumoniae* not detected by automated susceptibility testing. Emerg Infect Dis 2006;12:1209-13.

Tsakris A, Poulou A, Pournaras S, Voulgari E, Vrioni G, Themeli-Digalaki K, Petropulou D, Sofianou D. A simple phenotypic method for the differentiation of metallo-beta-lactamases and class A KPC carbapenemases in Enterobacteriaceae clinical isolates. J Antimicrob Chemother 2010;65:1664-71.

Tzelepi E, Giakkoupi P, Sofianou D, Loukova V, Kemeroglou A, Tsakris A. Detection of extended-spectrum beta-lactamases in clinical isolates of *Enterobacter cloacae* and *Enterobacter aerogenes*. J Clin Microbiol 2000;38:542-6.

Vading M, Samuelsen Ø, Haldorsen B, Sundsfjord AS, Giske CG. Comparison of disk diffusion, Etest, and VITEK2 for detection of carbapenemase-producing *Klebsiella pneumoniae* with the EUCAST and CLSI breakpoint systems. CMI 2011;17:668-74.

Voets GM, Fluit AC, Scharringa J, Cohen Stuart J, Leverstein-van Hall MA. A set of multiplex PCRs for genotypic detection of extended-spectrum beta-lactamases, carbapenemases, plasmid-mediated AmpC beta-lactamases and OXA-beta-lactamases. Int J Antimicrob Agents 2011;37:356-9.

Walsh TR, Bolmström A, Qwärnström A, Gales A. Evaluation of a new Etest for detecting metallo-beta-lactamases in routine clinical testing. J Clin Microbiol 2002;40:2755-9.

Wiener-Well Y, Rudensky B, Yinnon AM, Kopuit P, Schlesinger Y, Broide E, Lachish T, Raveh D. Carriage rate of carbapenem-resistant *Klebsiella pneumoniae* in hospitalised patients during a national outbreak. J Hosp Infect 2010;74:344-9.

Woodford N, Dallow JW, Hill RLR, Palepou MFI, Pike R, Ward ME, Warner M, Livermore DM. Ertapenem resistance among *Klebsiella* and *Enterobacter* submitted in the UK to a reference laboratory. Int J Antimicrob Agents 2007;29:456-9.

Yagci D, Yoruk F, Azap A, Memikoglu O. Prevalence and risk factors for selection of quinolone-resistant *Escherichia coli* strains in fecal flora of patients receiving quinolone therapy. Antimicrob Agents Chemother 2009;53:1287-9.

Appendix D – Comment round

Acton DS, Tempelmans Plat-Sinnige MJ, van Wamel W, de Groot N, van Belkum A. Intestinal carriage of *Staphylococcus aureus*: how does its frequency compare with that of nasal carriage and what is its clinical impact? Eur J Clin Microbiol Infect Dis. 2009;28:115-27.

Böcher S, Middendorf B, Westh H, Mellmann A, Becker K, Skov R, Friedrich AW. Semi-selective broth improves screening for methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 2010;65:717-20.

Bruins MJ, Juffer P, Wolfhagen MJHM, Ruijs GJHM. Salt tolerance of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus*. J Clin Microbiol 2007;45:682-3.

Coello R, Kiménez J, García M, Arroyo P, Minguez D, Fernández C, Cruzet F, Gaspar C. Prospective study of infection, colonization and carriage of methicillin-resistant *Staphylococcus aureus* in an outbreak affecting 990 patients.

Dutch Workingparty on Infection Prevention. Measures to prevent transmission of highly resistant microorganisms (HRMO). 2005. Available at: http://www.wip.nl.

Dutch Workingparty on Infection Prevention. MRSA hospital. 2007. Available at: http://www.wip.nl.

EUCAST 2011. Available at: http://www.eucast.org

Falcone M, Mezzatesta ML, Perilli M, Forcella C, Giordano A, Cafiso V, Amicosante G, Stefani S, Venditti M. Infections with VIM-1 metallo-beta-lactamase-producing *Enterobacter cloacae* and their correlation with clinical outcome. J Clin Microbiol 2009;47:3514-9.

Grmek-Kosnik I, Ihan A, Dermota U, Rems M, Kosnik M, Jorn Kolmos H. Evaluation of separate vs pooled swab cultures, different media, broth enrichment and anatomical sites of screening for the detection of methicillin-resistant *Staphylococcus aureus* from clinical specimens. J Hosp Infect 2005;155-61.

Hart CA, Gibson MF. Comparative epidemiology of gentamicin-resistant enterobacteria: persistence of carriage and infection. J Clin Pathol 1982;35:452-7.

Jeyaratnam D, Gottlieb A, Ajoku U, French GL. Validation of the IDI-MRSA system for use on pooled nose, axilla, and groin swabs and single swabs from other screening sites. Diagn Microbiol Infect Dis 2008;61:1-5. (B)

Jones EM, Bowker KE, Cooke R, Marshall RJ, Reeves DS, MacGowan AP. Salt tolerance of EMRSA-16 and its effect on the sensitivity of screening cultures. J Hosp Infect 1997;35:59-62.

Kelley PG, Grabsch EA, Howden BP, Gao W, Grayson ML. Comparison of the Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) assay, BD GeneOhm MRSA assay, and culture for detection of nasal and cutaneous groin colonization by MRSA. J Clin Microbiol 2009;47:3769-72.

Kerremans JJ, Maaskant J, Verbrugh HA, van Leeuwen WB, Vos MC. Detection of methicillin-resistant *Staphylocoocus aureus* in a low-prevalence setting by polymerase chain reaction with a selective enrichment broth. Diagn Microbiol Infect Dis 2008;61:396-401.

Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 1997;10:505-20.

Lauderdale TL, Wang JT, Lee WS, Huang JH, McDonald LC, Huang IW, Chang SC. Carriage rates of methicillin-resistant *Staphylococcus aureus* (MRSA) depend on anatomic location, the number of sites cultured, culture methods, and the distribution of clonotypes. Eur J Clin Microbiol Infect Dis 2010;29:1553-9.

Lautenbach E, Harris AD, Perencevich EN, Nachamkin I, Tolomea P, Metlay JP. Test characteristics of perirectal and rectal swab compared to stool sample for detection of fluoroquinolone-resistant *Escherichia coli* in the gastrointestinal tract. Antimicrob Agents Chemother 2005;49:798-800.

Luteijn JM, Hubben GAA, 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;17:146-54.

Malhotra-Kumar S, Abrahantes JC, Sabiiti W, Lammens C, Vercauteren G, Ieven M, Molenberghs G, Aerts M, Goossens H, on behalf of the MOSAR WP2 Study Team. Evaluation of chromogenic media for detection of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 2010;48:1040-6.

Matheson A, Christie P, Stari T, Kavanagh K, Gould IM, Masterton R, Reilly JS. Nasal swab screening for methicillin-resistant *Staphylococcus aureus* – How well does it perform ? A cross-sectional study. Infect Control Hosp Epidemiol 2012;33:803-8.

McAllister SK, Albrecht VS, Fosheim GE, Lowery HK, Peters PJ, Gorwitz R, Guest JL, Hageman J, Mindley R, McDougal LK, Rimland D, Limbago B. Evaluation of the impact of direct plating, broth enrichment, and specimen source on recovery and diversity of methicillin-resistant *Staphylococcus aureus* isolates among HIV-infected outpatients. J Clin Microbiol 2011;49:4126-30.

Mertz D, Frei R, Jaussi B, Tietz A, Stebler C, Flückiger U, Widmer AF. Throat swabs are necessary to reliably detect carriers of *Staphylococcus aureus*. Clin Infect Dis 2007;45:475-7.

Pasteran F, Mendez T, Guerriero L, Rapoport M, Corso A. Sensitive screening tests for suspected class A carbapenemase production in species of Enterobacteriaceae. J Clin Microbiol 2009;47:1631-9.

Poirel L, Bernabeu S, Fontineau N, Podglajen I, Lawrence C, Nordmann P. Emergence of OXA-48-producing *Escherichia coli* clone ST38 in France. Antimicrob Agents Chemother 2011;55:4937-8.

Ridley M. Perineal carriage of *Staph. aureus*. Br Med J 1959;1:270-3.

Solberg CO. A study of carriers of *Staphylococcus aureus* with special regard to quantitative bacterial estimations. Acta Med Scand Suppl 1965;436:1-96.

Solberg CO. Spread of *Staphylococcus aureus* in hospitals: causes and prevention. Scand J Infect Dis 2000;32:587-95

Stichting Werkgroep Antibioticabeleid (SWAB). Optimaliseren van het antibioticabeleid in Nederland XII. Herziening SWAB richtlijn Behandeling MRSA dragers. 2012.

Svent-Kucina N, Pirs M, Mueller-Premru M, Cvitkovic-Spik V, Kofol R, Seme K. One-year experience with modified BD GeneOhm MRSA assay for detection of methicillin-resistant *Staphylococcus aureus* from pooled nasal, skin, and throat samples. Diagn Microbiol Infect Dis 2009;63:132-9.

Vandenbergh MFQ, Verbrugh HA. Carriage of *Staphylococcus aureus*: epidemiology and clinical relevance. J Lab Clin Med 1999;133:525-34.

Verkade E, Ferket M, Kluytmans J. Clinical evaluation of Oxoid *Brilliance* MRSA Agar in comparison with bioMérieux MRSA ID medium for detection of livestock-associated meticillin-resistant *Staphylococcus aureus*. J Med Microbiol 2011;60:905-8.

Wassenberg MWM, Kluytmans JAJW, Bosboom RW, Buiting AGM, van Elzakker EPM, Melchers WJG, Thijsen SFT, Troelstra A, Vandenbrouke-Grauls CMJE, Visser CE, Voss A, Wolffs PFG, Wulf MWH, van Zwet AA, de Wit GA, Bonten MM. Rapid diagnostic testing of methicillin-resistant *Staphylococcus aureus* carriage at different anatomical sites: costs and benefits of less extensive screening regimens. Clin Microbiol Infect 2011;17:1704-10.

Wertheim FL, Verveer J, Boelens HAM, van Belkum A, Verbrugh HA, Vos MC. Effect of mupirocin treatment on nasal, pharyngeal, and perineal carriage of *Staphylococcus aureus* in healthy adults.

Wiener-Well Y, Rudensky B, Yinnon AM, Kopuit P, Schlesinger Y, Broide E, Lachish T, Raveh D. Carriage rate of carbapenem-resistant *Klebsiella pneumoniae* in hospitalised patients during a national outbreak. J Hosp Infect 2010;74:344-9.

Yagci D, Yoruk F, Azap A, Memikoglu O. Prevalence and risk factors for selection of quinolone-resistant *Escherichia coli* strains in fecal flora of patients receiving quinolone therapy. Antimicrob Agents Chemother 2009;53:1287-9.

APPENDIX B – ABBREVIATIONS

APBA	3-AminoPhenylBoronic Acid
AFLP	Amplified Fragment Length Polymorphism
ATCC	American Type Culture Collection
BA	Boronic Acid derivatives
BORSA	Borderline Oxacillin-Resistant *Staphylococcus Aureus*
BSAC	British Society for Antimicrobial Chemotherapy
CLSI	Clinical and Laboratory Standards Institute
CPE	Carbapenemase-Producing Enterobacteriaceae
CRE	Carbapenemase-Resistant Enterobacteriaceae
DPA	DiPicolinic Acid
EDTA	EthyleneDiamineTetraacetic Acid
ESBL	Extended-Spectrum Beta-Lactamase
ESBL-E	Extended-Spectrum Beta-Lactamase-producing Enterobacteriaceae
ESGARS	ESCMID Study Group for Antibiotic Resistance Surveillance
EUCAST	European Committee on Antimicrobial Susceptibility Testing
HPA	Health protection Agency
HRE	Highly Resistant Enterobacteriaceae
HRMO	Highly Resistant MicroOrganisms
IV	IntraVenous
LIS	Laboratorium voor Infectieziekten en perinatale Screening (English: Laboratory for Infectious diseases and perinatal Screening)
MALDI-TOF	Matrix-Assisted Laser Desorption Ionisation-Time Of Flight
MBL	Metallo-Beta-Lactamase
McF	McFarland standard
MHA	Mueller-Hinton Agar
MIC	Minimum Inhibitory Concentration
MODSA	Moderately Resistant *Staphylococcus Aureus*
MRSA	Methicillin-Resistant *Staphylococcus Aureus*
NVMM	Nederlandse Vereniging voor Medische Microbiologie (English: Netherlands Society for Medical Microbiology)
PBA	PhenylBoronic Acid
PBP	Penicillin-Binding Protein
PCR	Polymerase Chain Reaction
PEG	Percutaneous Endoscopic Gastrostomy
PFGE	Pulsed Field Gel Electrophoresis
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (English: National Institute for Public Health and the Environment)
SKMS	Stiching Kwaliteitsgelden Medisch Specialisten

SRGA	Swedish Reference Group for Antibiotics
LTAT	Laboratory TurnAround Time
WIP	Werkgroep Infectie Preventie (English: Dutch Workingparty on Infection Prevention)

APPENDIX C – UNPUBLISHED DATA

Chapter 2 – *Staphylococcus aureus*

2.1 Methicillin resistance

Wassenberg MWM, unpublished data

Methods

- Prospective multicentre study, 14 hospitals
- December 2005 - May 2008
- MRSA screening method: conventional microbiological cultures, including broth enrichment
- n = 299 patients at high risk for MRSA carriage according to WIP guideline, with an intravenous (IV) line, drain or catheter

Results

MRSA carriage was detected in nine patients (3.0%; 9/299) (Table 1). No MRSA positive IV line cultures were reported. In two patients cultures of 'percutaneous material' showed MRSA (in one patient a percutaneous endoscopic gastrostomy (PEG) tube, and in one patient a drain), yet these patients were heavily colonized at other sites as well. There were no MRSA carriers detected at the catheter insertion site only.

Table 1. Diagnostic yield of culturing catheter insertion sites in patients at high risk for MRSA carriage

Screening site	Screened *n*	MRSA positive *n*	MRSA detected at catheter insertion site *n*	MRSA deteted at catheter insertion site ONLY *n*
IV line	171	2 (1.2%)	0 (0%)	0 (0%)
Other (drain, PEG)	193	7 (3.6%)	2 (1.0%)	0 (0%)
IV line or other	299	9 (3.0%)	2 (0.7%)	0 (0%)

PEG = percutaneous endoscopic gastrostomy

Conclusion of the authors

Culturing catheter insertion sites does not have an additional benefit in screening patients at high risk for MRSA carriage.

Chapter 5 – Enterobacteriaceae

5.1 Extended-spectrum beta-lactamases

Diederen BMW, unpublished data

Methods

- Faecal sample or rectal swab (n=514 samples / n=384 patients)
- ChromID ESBL screening agar (bioMérieux)

  Tryptic soy broth with ceftazidime (1 mg/L) (TSB-CZ)

  Tryptic soy broth with cefotaxim (1 mg/L) (TSB-CTX)

  Tryptic soy broth (TSB)

  Oxidase test
- Screening:
  - Method A: ChromID – direct
  - Method B: TSB-CZ, subculture on chromID ESBL
  - Method C: TSB-CTX, subculture on chromID ESBL
  - Method D: TSB, subculture on chromID ESBL
- Confirmation:
  - Combined disk: cefotaxim, ceftazidime, and cefepime; both alone and combined with clavulanic acid
  - PCR and sequencing
- Golden standard:

  ESBL-positive: ESBL-positive isolate cultured with either one of method A t/m D

Results

ESBL-positive isolates were cultured from 23 of 514 samples (4%) and 21 of 384 patients (5%).

Table 1. Performance of method A - D

Growth of ONGNR	**ESBL-positive* *n***	**Sensitivity**	***p*-value****
ChromID ESBL	16	70%	
TSB + chromID ESBL	17	74%	n.s.
TSB-CZ + chromID ESBL	19	83%	n.s.
TSB-CTX + chromID ESBL	19	83%	n.s.
TSB-CZ + chromID ESBL *and/or* TSB-CTX + chromID ESBL	22	96%	<0,05
Total number of samples	*23*		

* according to golden standard; ** as compared to chromID ESBL; ONGNR = oxidase-negative gram-negative rod; n.s. = not significant.

Conclusions of the authors

The use of pre-enrichment (TSB-CZ, TSB-CTX, or TSB) results in an increased sensitivity of the chromID ESBL screeningsagar, although not statistically significant. Only a combination of two selective enrichment broths (TSB-CZ and TSB-CTX) yielded a statistically significant increase in sensitivity.

Kluytmans JAJW, unpublished data

Methods

- Faecal samples (n=200; one sample per patient)
- EbSA screening agar (Cepheid)
  ChromID ESBL screening agar (bioMérieux)
  Tryptic soy broth with vancomycin (8 mg/l) en cefotaxime (0,25 mg/L) (TSB-VC)
  Oxidase test
- Method A: EbSA – direct
  Method B: ChromID ESBL – direct
  Method C: TSB-VC, subculture on EbSA
  Method D: TSB-VC, subculture on chromID ESBL
- Confirmation:
  - Etest ESBL
  - Combined disk: cefotaxim and ceftazidime; both alone and combined with clavulanic acid (only in case of indeterminate result of Etest ESBL)
- Golden standard:
  ESBL-positive: ESBL-positive isolate cultured with either one of method A t/m D
  ESBL-negative: no ESBL-positive isolate cultured with either one of method A t/m D

Results

ESBL-positive isolates were cultured from 18 of 200 samples (9%).

Table 1. Performance of method A: EbSA – direct

Growth of ONGNR on EbSA	**ESBL-positive***	**ESBL-negative**	***Total***
Positive	16	21	*37*
Negative	2	161	*163*
Total	*18*	*182*	*200*

* according to golden standard; ONGNR = oxidase-negative gram-negative rod

Sensitivity = 16/18 = 89%

Specificity = 161/182 = 89%

Positive predictive value = 16/37 = 43%

Negative predictive value = 161/163 = 99%

Table 2. Performance of method B: ChromID ESBL – direct			
Growth of ONGNR on EbSA	**ESBL-positive***	**ESBL-negative**	***Total***
Positive	15	33	*48*
Negative	3	149	*152*
Total	*18*	*182*	*200*

* according to golden standard; ONGNR = oxidase-negative gram-negative rod

Sensitivity = 15/18 = 83%

Positive predictive value = 15/48 = 31%

Specificity = 149/182 = 82%

Negative predictive value = 149/152 = 98%

Table 3. Performance of method C: TSB-VC, subculture on EbSA			
Growth of ONGNR on EbSA	**ESBL-positive***	**ESBL-negative**	***Total***
Positive	18	10	*28*
Negative	0	172	*172*
Total	*18*	*182*	*200*

* according to golden standard; ONGNR = oxidase-negative gram-negative rod

Sensitivity = 18/18 = 100%

Positive predictive value = 18/28 = 64%

Specificity = 172/182 = 95%

Negative predictive value = 172/172 = 100%

Table 4. Performance of method D: TSB-VC, subculture on chromID ESBL			
Growth of ONGNR on EbSA	**ESBL-positive***	**ESBL-negative**	***Total***
Positive	18	22	*40*
Negative	0	160	*160*
Total	*18*	*182*	*200*

* according to golden standard; ONGNR = oxidase-negative gram-negative rod

Sensitivity = 18/18 = 100%

Positive predictive value = 18/40 = 45%

Specificity = 160/182 = 88%

Negative predictive value = 160/160 = 100%

Table 5. Comparison of performance of method A – D				
	Sensitivity	**Specificity**	**Positive predictive value**	**Negative predictive value**
EbSA	89%	89%	43%	99%
ChromID ESBL	83%	82%	31%	98%
TSB-VC + EbSA	100%	95%	64%	100%
TSB-VC + chromID ESBL	100%	88%	45%	100%

Conclusion of the authors

The use of a selective pre-enrichment (TSB-VC) increases both the sensitivity and the specificity of the EbSA and chromID ESBL screenings agars.

Performance of the Dutch national phenotypic ESBL detection guideline in the clinical setting

T.N. Platteel[1,2], J.W. Cohen Stuart[2], G.M. Voets[2], J. Scharringa[2], A.J. de Neeling[3], N. van de Sande[3], M.J.M. Bonten[2], A.C. Fluit[2], M.A. Leverstein-Van Hall[2,3]
On behalf of the ESBL national surveillance working group.

University Medical Center Utrecht

1) SALTRO, Dept. of Medical Microbiology, Utrecht, The Netherlands. 2) University Medical Centre Utrecht, Dept. of Medical Microbiology, Utrecht, The Netherlands. 3) National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control, Bilthoven, The Netherlands.

Introduction

In 2008, the Dutch Society for Medical Microbiology (NVMM) formulated and implemented a guideline for phenotypic screening and confirmation of ESBLs (Extended-Spectrum ß-Lactamases) in Enterobacteriaceae.
Confirmation of ESBL production is performed with ESBL Etest or combination disc (CD) in Enterobacteriaceae with a positive screentest (ceftazidime/ceftriaxone MIC>1 mg/L or an ESBL alarm by Phoenix or Vitek-2).

Aims

The aims of this study were
1. To determine the accuracy of phenotypic ESBL detection in Dutch clinical laboratories using this guideline.
2. To compare performance of Etest and CD as ESBL confirmation tests in the clinical setting.

Figure 1. Participating laboratories

Methods

• In 2009, 20 laboratories (Figure 1) submitted all *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Proteus mirabilis* and *Enterobacter* spp isolates with a positive ESBL screen test to a reference laboratory (n=463), of which 70% harboured an ESBL gene.
The first 25 non-repeat isolates per laboratory were included.

• Genotypic detection of ESBLs using microarray analysis and sequencing was used as reference test.

• Phenotypic confirmation tests were centrally repeated in case of a discrepant result of the phenotype reported by the participating laboratory and the ESBL genotype.

Results

Evaluation of Screening step

• The PPV of ESBL screening with automated systems was 70% (versus 92% with disc diffusion, performed in only one lab).

Table 1. Comparison of ESBL confirmation with Etest versus combination disc in the clinical setting.

	All isolates n=443* ESBL-pos n=312 ESBL-neg n=131	**Etest n=308 ESBL-pos n=221 ESBL-neg n=87**	**CD n=161 ESBL-pos n=113 ESBL-neg n=48**	**p-value (Etest vs CD)**
Sensitivity	95% (296/312)	96% (212/221)	93% (105/113)	N.S.
Specificity	70% (92/131)	59% (51/87)**	92% (44/48)	P<0.001
PPV	93% (296/320)	91% (212/233)	96% (105/109)	N.S.
NPV	90% (92/102)	94% (51/54)	85% (44/52)	N.S.

* N=443 (Etest 282, combination disc 135, both Etest and combination disc 26 isolates)
** The participating laboratories reported the ESBL Etest as off-range in 15 of 87 (17%) of the ESBL-negative isolates and false-positive in 21 of 87 (24%) of the ESBL-negative isolates.
N.S.: not significant.

Table 2. Performance of ESBL confirmation in Enterobacteriaceae without (group I) and with inducible chromosomal AmpC beta-lactamase (group II) in the clinical setting.

	GroupI (n=389) ***E. coli*** ***K. pneumoniae*** ***P. mirabilis*** ***K. oxytoca***	***E. coli* n=326 ESBL pos n=248 (76%)**	***K. pneumoniae* n=37 ESBL pos n=35 (95%)**	***P. mirabilis* n=15 ESBL pos n=2 (13%)**	***K. oxytoca* n=11 ESBL pos n=3 (27%)**	**Group II (n=54)** ***E. cloacae*** **ESBL pos n=24 (44%)**	**p-value for comparison group I versus group II**
Sensitivity	96% (276/288)	96% (238/248)	94% (33/35)	100% (2/2)	100% (3/3)	83% (20/24)	p=0.026
Specificity	65% (66/101)	69% (54/78)	50% (1/2)*	62% (8/13)	38% (3/8)	87% (26/30)	p=0.03
PPV	92% (276/299)	95% (238/251)	97% (33/34)	33% (2/6)	38% (3/8)	95% (20/21)	N.S.
NPV	90% (66/73)	90% (54/60)	50% (1/2)*	100% (8/8)	100% (3/3)	90% (26/29)	N.S.

* Only 2 ESBL-negative *K. pneumoniae* isolates were included, one of which was incorrectly reported as false-positive in the ESBL confirmation test.
N.S.: not significant

• The PPV of ESBL screening varied per species from 95% for *K. pneumoniae* versus 13-76% in the other Enterobacteriaceae.

•The PPV of screening correlated with MIC of 3rd generation cephalosporins (measured in central lab by broth microdilution)
cefotaxime and ceftazidime < 2mg/L (n=43 isolates, 10%): PPV was 9%**
cefotaximee and ceftazidime ≥ 2mg/L (n=400 isolates, 90%): PPV was 77% (p<0.001)

** 50% of false-positive confirmation results were from this category.

Phenotypic ESBL confirmation

• Test characteristics of ESBL confirmation performed in the participating labs are shown in Table 1 and Table 2.

• The specificity of ESBL confirmation would increase from 70% (296/312) to 80% (105/131) and the sensitivity would decrease from 95% (296/312) to 94% (293/312) if ESBL confirmation is exclusively performed in isolates which meet the MIC or zone diameter ESBL screen criteria of the indicator cephalosporins, i.e. when isolates with a low cephalosporin MIC but an automated system ESBL alarm are not considered ESBL screen positive.

• 7% of the ESBL confirmation tests were misinterpreted by the participating laboratories (8% Etests versus 4% CD; p>0.05)

• In case of a positive screentest:
1. In *K. pneumoniae*, the diagnostic yield of ESBL confirmation is limited.
2. For *E. coli* and *Enterobacter* spp., ESBL confirmation is required.
3. For *P. mirabilis* and *K. oxytoca*, a negative confirmation test result excludes ESBL production, whereas a positive result necessitates genotypic ESBL detection.

• Sensitivity, PPV and NPV of Etest and CD were not statistically different. CD was more specific than Etest (92% vs 59%), due to the non-determinable and false-positive Etest results.

Conclusions

• Implementation of a national guideline has resulted in adequate phenotypic detection of ESBL-positive isolates except for *P. mirabilis* and *K. oxytoca*.

• The PPV of ESBL screening was dependent on species and MIC.

• Although the sensitivity, PPV and NPV of confirmation with CD and Etest were comparable, Etests were less specific due to non-determinable and false-positive results.

• Education of laboratory employees may improve Etest interpretation.

• If ESBL confirmation is exclusively interpreted in isolates which meet the MIC or zone diameter ESBL screen criteria of the indicator cephalosporins, specificity is increased by 10% while sensitivity is reduced only 1%

E-mail: t.platteel@umcutrecht.nl

Screening and confirmation methods for carbapenemases in Enterobacteriaceae

James Cohen Stuart, Guido Voets, Sebastiaan Voskuil, Ad Fluit, Jelle Scharringa, Maurine Leverstein-van Hall.

Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands

University Medical Center Utrecht

Introduction

In 2010, a guideline for detection of carbapenemases in Enterobacteriaceae was published on behalf of the Dutch working party on detection of highly resistant micro-organisms (Cohen Stuart et al, IJAA 2010).
This guideline recommends a meropenem screening breakpoint for all Enterobacteriaceae of ≥0.5 mg/L or a meropenem zone diameter of ≤23mm (10ug disc loading).
For *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Salmonella* spp. and *Citrobacter* spp. an imipenem screening breakpoint of ≥2mg/L was recommended or a zone diameter of ≤21mm (10ug disc loading).
Ertapenem ≥0.5 mg/L was not recommended because of indications in the literature that it is less specific for carbapenemase production.
Carbapenemase inhibition tests with boronic acid (BA) were recommended for KPC and with EDTA or dipicolinic acid (DPA) for metallo-carbapenemases (MBL).

Objectives

1. To compare meropenem and ertapenem as screening carbapenems for carbapenemase production.
2. To compare the breakpoints proposed in the Dutch guideline with EUCAST clinical breakpoints.
3. To evaluate the inhibition tests with BA and DPA for confirmation of carbapenemase production.
4. To evaluate the CICA-Beta-Test as MBL detection test.

Methods

Isolates
* 61 carbapenemase producers:
  30 KPC, 25 MBL (16 VIM, 6 GIM, 3 NDM), 4 KPC plus VIM, 1 SME, 1 OXA-48.
  44 *K. pneumoniae*, 7 *E. coli*, 5 *Enterobacter* spp., 3 *S. marcesens*, 2 *P. mirabilis*

* 136 carbapenemase negative controls:
  32 AmpC, 84 ESBL, 3 ESBL/plasmid AmpC combined, 8 K1 K. oxytoca, 2 non ESBL TEM/SHV, 7 E. coli without beta-lactamase)

Determination of MIC
* MICs of meropenem and ertapenem were determined using broth micro-dilution (Merlin).

Disc diffusion
Meropenem zone diameters were determined using 10ug discs (Mast, UK) and 10 ug tablets (Rosco, Danmark) on MHA-agar (Becton Dickinson).

Phenotypic carbapenemase confirmation tests:
The following combination tablets (Rosco, Danmark) were used:
1 meropenem plus amino-phenyl boronic acid (APBA)
2 meropenem plus DPA
3 meropenem plus cloxacillin
4 meroepenem plus DPA plus APBA

Zone diameter criteria for KPC production:
Increase of zone ≥ 5mm with APBA plus an increase of zone ≤ 5 mm with cloxacillin compared to meropenem alone.

Zone diameter criteria for MBL production:
Increase of zone ≥ 5mm with DPA compared to meropenem alone.

Cica-Beta Tests
Cica-Beta-Tests were obtained form Mast.

Detection of beta-lactamase genes
Detection of beta-lactamase genes (ESBL, plasmid AmpC, carbapenemases) was performed using PCR and sequencing, as described by Voets et al (IJAA 2010).

Results of screening for carbapenemase production:

* Screening with meropenem is more sensitive and specific than with ertapenem, as shown in table 1.
* Meropenem and ertapenem MICs are shown in Figures 1 and 2.
* Carbapenemase screening with the clinical EUCAST breakpoint of meropenem has 92% sensitivity versus 100% with the meropenem ≥0.5 mg/L screening breakpoint.

Table 1: test characteristics of screening with meropenem and ertapenem.

	Dutch Carbapenemase Detection Guideline				EUCAST Clinical Breakpoints	
	meropenem ≥0.5 mg/L	meropenem disc (10µg, Mast) ≤ 23 mm	meropenem tablet (10µg, Rosco) ≤ 23 mm	ertapenem ≥0.5 mg/L	meropenem I/R (≥4 mg/L)	ertapenem I/R (≥1 mg/L)
Sensitivity	100%	100%	97%	92%[1]	92%[1]	89%[3,2]
Specificity	96%	99%	98%	76%[2]	99%[1]	83%[3]

1 comparison with meropenem ≥0.5 mg/L not significant.
2 p<0.001 for comparing specificity with meropenem ≥0.5 mg/L.
3 Comparison with ertapenem ≥0.5 mg/L not significant.

Figure 1: meropenem MIC of isolates.

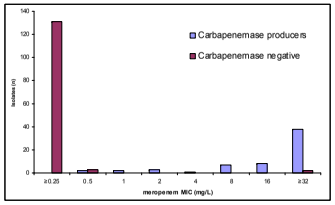
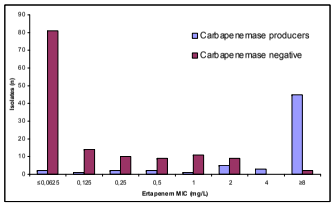


Figure 2: ertapenem MIC of isolates.



Results of phenotypic confirmation tests

* Overall sensitivity of carbapenemase detection was 92%
* For KPC, the sensitivity was 97% and the specificity 100% (33/34,Table 2)
* For MBL, the sensitivity was 93% (27/29), and the specificity 100%
* The SME and OXA-48 producing isolates showed no synergy with DPA or BA.
* The sensitivity of the Cica-Beta tests for MBL detection was 41% and the specificity of 34% (data not shown).

Table 2: Results of inhibition tests

Carbapenemase (inhibitor)	n	sensitivity	specificity
KPC (APBA)	30	29/30 (97%)	136/136 (100%)
MBL (DPA)	25	23/25 (92%)	136/136 (100%)
KPC plus MBL (DPA plus APBA)	4	4/4 (100%)	136/136 (100%)

Conclusions

1. For carbapenemase detection, screening with meropenem is more sensitive and specific than screening with ertapenem.

2. The meropenem ≥0.5 mg/L screening breakpoint for carbapenemases was 100% sensitive versus 92% using the meropenem EUCAST clinical breakpoint.

3. The inhibition tests with BA for KPC and with DPA for MBLs are sensitive and specific.

4. Cica-Beta strips require further development for detection of MBLs.

Acknowledgements

Vivi Miriagou, Collin MacKenzie for providing carbapenemase producing isolates.

APPENDIX D – COMMENT ROUND

CONCEPT VERSION 0.1

COMMENT 1

Author

Dr. J. Kalpoe, *Slotervaart Hospital / Netherlands Cancer Institute (Amsterdam)*

Chapter, paragraph, page and line concerned

Chapter 1 – General introduction / 1.7 Methods / 1.7.2 Procedures working group / page 7, line 37-39

Comment

Appendix D ontbreekt, maar dat komt wellicht in de finale versie?

Response

The current final draft guideline has not been sent to international experts. Therefore, appendix D is indeed not included, and should not have been referred to. The working group leaves the decision to ask international experts for their comment to the discretion of the Quality Committee of the Netherlands Society for Medical Microbiology (NVMM).

Changes

- Table of contents / page 4, line 4: 'Appendix D – External referents' has been deleted
- Table of contents / page 4, line 5: 'Appendix E – Comment round' has been changed in 'Appendix D – Comment round'
- Chapter 1 – General introduction / 1.7 Methods / 1.7.2 Procedures working group / page 7, line 37-39: 'The final draft guideline … (see Appendix D).' has been deleted
- Chapter 1 – General introduction / 1.8 Authorisation and implementation / page 8, line 16: 'Appendix E' has been changed in 'Appendix D'

COMMENT 2

Author

Dr. J. Kalpoe, *Slotervaart Hospital / Netherlands Cancer Institute (Amsterdam)*

Chapter, paragraph, page and line concerned

Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.2.1 Culture sites / page 9, line 29-31

Comment

Het is mij hieruit niet duidelijk of een perianal swab wel of niet aanbevolen wordt als alternatief voor de rectal swab. Tekst in regel 29-30 suggereerd van wel, maar regel 31 juist weer niet.

Response

The working group considers the **perianal** and **perineal** regions as distinct culture sites, where a **perianal** swab is considered an acceptable non-invasive alternative to a rectal swab [Lautenbach 2005, Wiener-Well 2010] (line 29-30), but a **perineal** swab is not recommended (line 31).

Changes

No changes.

COMMENT 3

Author

Dr. J. Kalpoe, *Slotervaart Hospital / Netherlands Cancer Institute (Amsterdam)*

Chapter, paragraph, page and line concerned

Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.2.2 Number of cultures / page 10, line 3-10

Comment

Moet uit deze paragraaf geconcludeerd worden dat het geen zin heeft om "loss of carriage of HRE" tijdens dezelfde opname te identificeren? M.a.w.: moet de patient tijdens de zelfde opname als waarin HRE is gedetecteerd altijd als gekoloniseerd beschouwd worden en hebben screening kweken om "loss of carriage" te detecteren tijdens die opname dan geen zin?

Response

Since carriage of HRE may be prolonged, in particular in patients that are hospitalised and use antibiotics [Hart 1982, Yagci 2009], the working group does indeed take the view that it is not appropriate to take follow-up cultures to identify loss of carriage of HRE during hospitalisation.

Changes

No changes.

COMMENT 4

Author

Dr. A. van Griethuysen, *Rijnstate (Arnhem)*

Chapter, paragraph, page and line concerned

Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.3.5 Susceptibility testing / page 12, line 3-5; page 13, line 1, 11, and 15; page 19, line 7 and 9

Comment

Het is makkelijker om de diskdiffusie breekpunten conform de EUCAST te presenteren, dwz een screeningsbreekpunt voor meropenem < 24 ipv <= 23 en voor imipenem <22 ipv <=21.

Response

The working group agrees with the comment.

Changes

- Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.3.5 Susceptibility testing / page 12, line 3: ≤ 23 replaced with < 24
- Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.3.5 Susceptibility testing / page 12, line 4-5: ≤ 21 replaced with < 22
- Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.3.5 Susceptibility testing / page 13, line 1: ≤ 23 replaced with < 24
- Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.3.5 Susceptibility testing / page 13, line 11 and 15: ≤ 21 replaced with < 22
- Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.6 Recommendations / page 19, line 7: ≤ 23 replaced with < 24
- Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.6 Recommendations / page 19, line 9: ≤ 21 replaced with < 22

COMMENT 5

Author

Dr. J. Kalpoe, *Slotervaart Hospital / Netherlands Cancer Institute (Amsterdam)*

Chapter, paragraph, page and line concerned

Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.7.1 Time-to-result / page 20, line 3-4

Comment

Mag hieruit opgemaakt worden dat de maximale "time to result" van CPE screening 48 uur is en van bevestiging 72 uur? (regel 4 pagina 20). Verder: wat wordt bedoeld met een "mean time-to-result" (o.a regel 3-4 pagina 20), wat is de SD dan? mag dat plus/min 1 a 2 dagen zijn? Zou het niet duidelijker zijn als er van een maximale "mean time to result" wordt gesproken?

Response

See comment 7.

Changes

See comment 7.

COMMENT 6

Author

Quality Committee, *Netherlands Society for Medical Microbiology*

Chapter, paragraph, page and line concerned

Title page, line 15

Comment

Bij Prof. Dr. M.J.M. Bonten ontbreekt een toevoeging van de professie clinical microbiologist is dit bewust? Het geeft onduidelijkheid.

Response

The profession of Prof. dr. M.J.M. Bonten was inadvertently not listed on the title page.

Changes

- Title page, line 15: 'Prof. dr. M.J.M. Bonten' has been changed in 'Prof. dr. M.J.M. Bonten, clinical microbiologist'.

COMMENT 7

Author

Quality Committee, *Netherlands Society for Medical Microbiology*

Chapter, paragraph, page and line concerned

Chapter 1 – General introduction / 1.6 Methods / 1.6.3 Quality indicators / page 7, line 20-22
Chapter 5 – Enterobacteriaceae / 5.1 Extended-spectrum beta-lactamases / 5.1.6 Quality indicators / page 19, line 7-11
Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.7 Quality indicators / page 30, line 38 – page 31, line 11

Comment

In paragraaf 5.1.6 en 5.3.7 staan 'quality indicators' genoemd. De CK is van mening dat de huidige beschrijving van deze 'quality indicators' nog onvoldoende uitgewerkt is. Wij stellen voor de normen en eventueel prestatie-indicatoren op te nemen in de definitieve versie en nu achterwege te laten. Hieronder een overzicht van de vragen en overwegingen naar aanleiding van deze indicator. De in de richtlijn gekozen prestatie-indicator is de procesindicator time-to-result. Deze benadering gaat uit van de aanname dat kwaliteit (op het nivo van de patiënt) verbetert naarmate het resultaat van de uitslag sneller beschikbaar is. Hoe onderbouwd is deze aanname en in hoeverre is de keuze voor een procesindicator time-to-result een risico voor de kwaliteit van de daadwerkelijke uitkomst (wel/niet

resistentie aanwezig, onterecht wel/niet opsturen, onterecht wel/niet isoleren of intensief behandelen)? Het is onze aanname dat de factor frequentie van voorkomen in een laboratorium hier eveneens een rol in speelt (incidenteel versus uitbraak gerelateerd). Wordt met deze benadering de juiste prestatieprikkel gegeven?
In de paragrafen 5.1.6 en 5.3.7 staan vervolgens normen genoemd gerelateerd aan deze procesindicator. Wij pleiten voor het maken van een onderscheid tussen enerzijds de procesindicator en anderzijds de norm. In de huidige tekst is het verschil tussen indicator en norm niet onderscheidend geformuleerd en wordt gesproken over een standaard. Daarnaast is niet benoemd welk soort norm dit is, een minimum norm of een streefnorm? Opvallend is ook dat in 5.3.7 twee verschillende normen worden gehanteerd, in lab A mag het gemiddeld 5 dagen duren, in lab B gemiddeld 9 dagen. Wat zegt dit dan uiteindelijk over deze normen in relatie met de kwaliteit op het nivo van de patiëntenzorg? Normen en prestatie-indicatoren dienen een onderdeel te zijn van het gehele kwaliteitsbeleid. Opname in een richtlijn betekent dat een prestatie-indicator uitgevraagd kan worden in de audit van de laboratoria en vakgroepen door derden waaronder IGZ. Laboratoria dienen in dat geval dan ook een registratie bij te houden van de betreffende indicator. Deze registratie dient uitvoerbaar en in balans met de beoogde kwaliteitsverbetering te zijn en dus zorgvuldig gekozen te worden.

Response

The working group agrees with the comment to withhold the quality indicators from the current version of the guideline.

Changes

- Chapter 1 – General introduction / 1.6 Methods / 1.6.2 Aspects of laboratory detection / page 7, line 17-17: ‘and 5) quality indicators: time-to-result’ has been deleted.
- Chapter 1 – General introduction / 1.6 Methods / 1.6.3 Quality indicators / page 7, line 19-22: Paragraph ‘1.6.3 Quality indicators’ has been deleted.
- Chapter 5 – Enterobacteriaceae / 5.1 Extended-spectrum beta-lactamases / 5.1.6 Quality indicators / page 19, line 7-11: Paragraph ‘5.1.6 Quality indicators’ has been deleted.
- Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.7 Quality indicators / page 30, line 38 – page 31, line 11: Paragraph ‘5.3.7 Quality indicators’ has been deleted.

COMMENT 8

Author

Quality Committee, *Netherlands Society for Medical Microbiology*

Chapter, paragraph, page and line concerned

Chapter 1 – General introduction / 1.9 Revision / page 8, line 25-27

Comment

In de huidige beschrijving is gekozen de beoordeling voor de noodzaak voor revisie bij het NVMM bestuur te leggen. De CK heeft voorkeur voor het in stand houden van de werkgroep voor een periode van 5 jaar en deze werkgroep verantwoordelijk te maken voor de beoordeling voor de noodzaak voor revisie. Zo is het beschreven in het 'Protocol voor de ontwikkeling, autorisatie en revisie van beroepsgebonden richtlijnen van de NVMM'. De argumentatie hiervoor is dat de werkgroepleden bij uitstek actief zin in het onderwerp en dus logischerwijs als eerste op de hoogte zijn van relevante aanpassingen meer dan de bestuursleden van de NVMM. Hiermee blijven de verantwoordelijkheden ten aanzien van de revisie behapbaar, immers verdeeld over de diverse werkgroepen.

Response

The working group agrees with the comment.

Changes

- Chapter 1 – General introduction / 1.9 Revision / page 8, line 25: 'NVMM' has been changed into 'working group'.
- Chapter 1 – General introduction / 1.9 Revision / page 8, line 27: 'board of the NVMM' has been changed into 'working group'

COMMENT 9

Author

Dr. M. Hoogewerf, Drs. M. Scholing. Dr. A.P. van Dam, *OLVG (Amsterdam)*

Chapter, paragraph, page and line concerned

Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.3.5 Susceptibility testing / page 23, line 18 – page 24, line 15

Comment

Wij hebben recent een validatiestudie verricht ivm de detectie van carbapenemases in ons laboratorium. Hiervoor werden 9 carbapenemase-producerende stammen gebruikt (allen *Klebsiella pneumoniae*, 4 KPC/ 3 OXA-48/ 1 VIM-1/ 1 NDM-1) en 14 controle-stammen in de vorm van carbapenemase negatieve ESBL-producerende stammen (*Klebsiella pneumoniae* en *E coli*). We hebben oa de disk-diffusiemethode getest voor de screening.

T.a.v meropenem:

In onze studie werden van 9 geteste carbapenemase-stammen 3 stammen niet gedetecteerd bij een zonebreekpunt van ≤23mm voor meropenem 10 ug (allen OXA-48 stammen). Deze werden wel gedetecteerd bij een afkappunt van ≤27 mm. Hierdoor steeg de sensitiviteit van 70 naar 100%.
De specificiteit bleef 100% (geen van de 14 ESBL-producerende stammen met een afkappunt van ≤27 mm). Buiten de validatiestudie om hebben wij nog een andere carbapenemase-positieve *Klebsiella pneumoniae*-stam (OXA-48) in ons lab gedetecteerd, ook deze stam had een zone-

diameter van >23 mm (27 mm) en zou je niet met het in deze concept-richtlijn voorgestelde screenings-afkappunt detecteren.

T.a.v. imipenem:

We hebben van dezelfde stammen ook zone-diameters rond de imipenem disk (10 ug) gemeten. Bij 4/9 carbapenemase-producerende stammen werd een zone-diameter van > 21 mm gemeten (tot 25 mm, 1 KPC-2 en 3 OXA-48 stammen). De sensitiviteit van deze screeningstest bedroeg dus 56 %. Overigens hebben we bij de later in ons lab gedetecteerde carbapenemase-stam (OXA-48) ook een zone diameter van > 21 mm gemeten (26 mm).

Concluderend hebben wij vastgesteld dat met de huidige geadviseerde screenings-afkapwaarden voor de zone diameters rond zowel de meropenem als imipenem disk een onacceptabel hoog aantal carbapenemases gemist wordt. Het gaat met name om de OXA-48 stammen, maar er werd ook een KPC-stam gemist. Daarom adviseren wij om bij een zone-meting rond de meropenem disk (maar ook rond de imipenem disk) een grotere diameter als breekpunt aan te houden om de sensitiviteit te verhogen. Wij houden rond de meropenem disk zelf een breekpunt aan van ≤ 27 mm, dit levert een hogere sensitiviteit op en in ons validatie-onderzoek geen fout-positieven (weliswaar alleen *K. pneumoniae*-stammen en een klein aantal). Wij adviseren de imipenem disk niet mee te nemen in de screening, omdat bij het aanhouden van een screeningszone van ≤ 27 mm rond de meropenem disk waarschijnlijk een zeer hoog aantal van de carbapenemase-producerende stammen wel wordt gedetecteerd [Pasteran 2009]. Bovendien ondervang je hiermee het probleem dat een imipenem screening afkappunt niet kan worden gebruikt voor een groot aantal *Enterobacteriaceae (Proteus spp., Serratia spp. Providencia spp. en Morganella morganii)*. Op basis van de aangegeven literatuur is het o.i. in het algemeen twijfelachtig of er naast een algemene screening voor meropenem ook een screening voor imipenem nodig is.

Response

The recommended zone diameter screening breakpoints have been based on 1) the zone diameter distribution of the wild-type population, i.e. screening breakpoint above the epidemiological cut-off [EUCAST 2011]; and 2) the zone diameters described in the literature for strains shown to have a carbapenemase gene. An increase in the zone diameter breakpoint would result in false-positive test results, not only in *K. pneumonia* and *E. coli*, but also in group II Enterobacteriaceae [EUCAST 2011]. The guideline clearly states that the meropenem zone diameter screening breakpoint is less sensitive than the MIC screening breakpoint (84-97% vs. 100%) [Pasteran 2009, Cohen Stuart *unpublished data*], and that sporadic VIM-producers and some OXA-48-producing isolates will not be detected using meropenem screening breakpoints [Falcone 2009, Poirel 2011]. In case of an outbreak of OXA-48 producing Enterobacteriaceae it is, therefore, recommended to use ertapenem for screening [Poirel 2011].

First, it can not be read from the data presented by the authors from the OLVG whether the use of the meropenem **MIC** screening breakpoint would have resulted in the same sensitivity as that observed for the **zone diameter** screening breakpoint. Second, the data presented pertain to a limited number

of isolates (n=9 carbapenemase-positive and n=14 carbapenemase-negative), where it is unclear whether the three OXA-48-producing isolates are clonally related or not.
In conclusion, the working group considers the data presented too limited to justify an increase in the meropenem zone diameter breakpoint at this moment. However, the validity of the recommended screening breakpoints will be regularly reconsidered against the background of actual European and national antimicrobial resistance surveillance data.

Changes
No changes.

COMMENT 10

Author
Quality Committee, *Netherlands Society for Medical Microbiology*

Chapter, paragraph, page and line concerned
Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.5.3 Surveillance / page 29, line 14-17

Comment
De CK maakt bezwaar tegen de door de werkgroep gekozen procedure ten aanzien van het registratiebeleid. Richtlijnen die voor autorisatie aangeboden worden op een ALV dienen vast te staan en worden als besluitvormend geagendeerd. Dat wil zeggen dat er geen discussiepunten meer open staan. De CK verzoekt u dringend tot consensus te komen voorafgaand aan de ALV en in de richtlijn een heldere formulering op te nemen ten aanzien van surveillance. Een meer generaliserende benadering van het onderwerp waarbij gewezen wordt op het nut van surveillance en registratie en de benoeming van de plichten van alle partijen hierin (RIVM en laboratoria) zonder dat een expliciete vorm ingevuld wordt biedt hier mogelijk uitkomst? Het is voorstelbaar dat de vorm in de tijd verandert (van 1 centraal laboratorium naar meer of andersom al naar gelang praktijkervaring en epidemiologie).

Response
The working group agrees with the comment.

Changes
- Chapter 1 – General introduction: The following paragraph has been added ‘1.6.3 Surveillance of resistance: Although the surveillance of antimicrobial resistance is not part of this guideline on the laboratory detection of HRMO, the working group does recommend medical microbiology laboratories to participate in national surveillance programs that aim on the monitoring and early detection of trends in antimicrobial resistance on a national level.’.
- Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.5.3 Surveillance / page 29, line 14-17: Paragraph ‘5.3.5.3 Surveillance’ has been deleted.

CHANGES MADE ON BEHALF OF MEMBERS OF THE WORKING GROUP

The following changes have been made on behalf of members of the working group:

- Chapter 1 – General introduction / 1.5 Working group / 1.5.2 Conflict of interest / page 6, line 32-33: Disclosures of potential conflict of interest by working group members have been added.
- Chapter 5 – Enterobacteriaceae / 5.1 Extended-spectrum beta-lactamases / 5.1.4 Reporting / page 17, line 24: 'OR' has been changed in 'AND'.
- Chapter 5 – Enterobacteriaceae / 5.1 Extended-spectrum beta-lactamases / 5.1.5 Recommendations / page 19, line 2: 'OR' has been changed in 'AND'.
- Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.3.5 Susceptibility testing / page 23, Figure 1: 'meropenem or imipenem Etest' has been changed in 'antibiotic gradient on a strip method'.
- Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.3.5 Susceptibility testing / page 23, Figure 1: '4: Imipenem-EDTA Etest' has been changed into: '5: Imipenem-EDTA Etest'.
- Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.3.5 Susceptibility testing / page 25, line 1: 'Etest' has been changed in 'an antibiotic gradient on a strip method (e.g. Etest)'.
- Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.3.5 Susceptibility testing / page 26, line 27: 'Etest' has been changed in 'an antibiotic gradient on a strip method (e.g. Etest)'.
- Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.5 Reporting / page 29, line 9: 'OR' has been changed in 'AND'.
- Chapter 5 – Enterobacteriaceae / 5.3 Carbapenemases / 5.3.6 Recommendations / page 30, line 33: 'OR' has been changed in 'AND'.

CONCEPT VERSION 1.1

COMMENT 11

Author

Dr. B. Postma, Dr. E.M. Mascini, Drs. T-N. Le, Drs. R.W. Bosboom, Dr. A.A. van Zwet, Dr. A.J. van Griethuysen, Dr. M.A. Schouten, Dr. C.M.A. Swanink, Drs. J. Keijman, *Maatschap Medische Microbiologie & Immunologie Gelderland (Arnhem)*

Chapter, paragraph, page and line concerned

Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin-resistance / 2.1.2.1 Culture sites / page 10, line 24-25

Comment

In de conceptrichtlijn wordt aangegeven om bij screening van personeel neus, keel én perineum te kweken. De vigerende richtlijn geeft aan om alleen neus en keel te kweken en pas bij aanhoudende problemen van verspreiding een perineumkweek toe te voegen.

In de ziekenhuizen die onze maatschao bedient hebben wij ruime ervaring met MRSA-uitbraken. Het is echter nog nooit nodig geweest om perineumkweken af te nemen bij medewerkers om deze uitbraken onder controle te brengen. Wij maken daarom bezwaar tegen deze aanpassing in verband met een hogere belasting en een lage compliance indien er standaard een perineumkweek wordt gevraagd. Is het de werkgroep bekend hoe vaak uitbraken voorkomen die pas onder controle kwamen nadat ook perineumkweken werden afgenomen en is er een afweging gemaakt of dit een wijziging in beleid noodzakelijk maakt?

Response

The nose, more specifically the vestibulum nasi, is the most important carriage site of *S. aureus* [Kluytmans 1997, Matheson 2012, Vandenbergh 1999]. However, *S. aureus* can be isolated from other body sites, and not all carriers do carry *S. aureus* in the nose [Acton 2009, Lauderdale 2010]. Perineal carriage of *S. aureus* is frequently observed, and in some carriers the only site of carriage. Studies in both patients and healthy adults have shown that a perineal swab, added to a nasal and throat swab, increases the yield of *S. aureus* detection in carriers with up to 14% [Acton 2009, Coello 1994, Lauderdale 2010, Matheson 2012, Wertheim 2005]. Perineal carriage is known for its potential to disperse large numbers of *S. aureus* into the environment [Ridley 1959, Solberg 1965, Solberg 2000]. In addition, the site of MRSA carriage affects the choice of eradication strategy. Perineal carriage is one of the criteria to define complicated MRSA carriage, which asks for a different eradication strategy than uncomplicated MRSA carriage [SWAB 2012]. In conclusion, the working group takes the view that the inclusion of a perineal swab as a recommended culture site for both patients and healthcare workers fits within the aim of this guideline, i.e. to provide recommendations on the optimal detection of carriage of highly resistant microorganisms, in this case MRSA.

Changes

- Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin-resistance / 2.1.21 Culture sites / page 10, line 32: The following references have been added: ‘Coello 1994’ en ‘ Wertheim 2005‘..

COMMENT 12

Author

Dr. G.A. Kampinga, namens artsen-microbioloog UMCG, *UMC Groningen (Groningen)*

Chapter, paragraph, page and line concerned

Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin-resistance / 2.1.3.2 Solid agar media / page 15, line 10-14

Comment

Er wordt gesteld dat het nodig is dat een bloedagar moet worden bijgeënt om te kijken of er groei is. Sinds juli 2011 werken we bij opgenomen patiënten met een MRSA-sneltest (PCR) met als doel onnodige isolaties zo kort mogelijk te houden. Het is dan niet logisch om de groei op een BA af te

moeten wachten om te bepalen of de kweek betrouwbaar is. We gaan er vanuit dat medewerkers in een ziekenhuis weten hoe ze uitstrijken moeten afnemen bij patiënten. We doen wel controle bloedagar bij zelf afgenomen kweken door patienten (bij thuiskweken) en bij medewerkers die kweken bij zichzelf afnemen. Bij medewerkers kunnen conflicterende belangen zijn omdat een positieve kweek voor hen nadelen kan hebben. Opgemerkt met worden dat als kweken van medewerkers met 1 kweekstok afgenomen mogen worden, een betrouwbare groeicontrole per locatie per definitie niet meer mogelijk is.

Response

As stated in paragraph 2.1.3 it is recommended to only use direct molecular detection methods in case of urgency to provisionally exclude MRSA carriage. Direct molecular test methods should only be used in addition to conventional methods, i.e. broth enrichment, MRSA screening agar, species identification and susceptibility testing. Herewith, the decision to discontinue pre-emptive isolation based on a negative direct molecular test result will always be provisional, as the final culture result depends on the results of the conventional culture. The final culture result will not be delayed by the result of the growth control. The use of a growth control is recommended for two reasons: 1) to disapprove MRSA cultures from non-sterile culture sites when the growth control is negative; and 2) as backup for MRSA isolates that are suppressed by the selective agents used in the MRSA screening agar.

Changes

No changes.

COMMENT 13

Author

Dr. B. Postma, Dr. E.M. Mascini, Drs. T-N. Le, Drs. R.W. Bosboom, Dr. A.A. van Zwet, Dr. A.J. van Griethuysen, Dr. M.A. Schouten, Dr. C.M.A. Swanink, Drs. J. Keijman, *Maatschap Medische Microbiologie & Immunologie Gelderland (Arnhem)*

Chapter, paragraph, page and line concerned

Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin-resistance / 2.1.3.2 Solid agar media / page 15, line 10-16

Comment

In de richtlijn wordt geadviseerd om naast de media voor MRSA screening ook een bloedplaat te enten als groeicontrole. Daarnaast wordt geadviseerd om kolonies op deze bloedplaat die verdacht zijn voor *S. aureus* verder te identificeren en een gevoeligheid te bepalen.
In onze ziekenhuizen worden de kweken altijd afgenomen door zorgprofessionals en wij gaan er dan ook vanuit dat dit correct gebeurt; vanuit dat oogpunt achten wij een groeicontrole niet nodig. Uw argument dat sommige stammen geremd zouden kunnen worden door de selectieve stoffen in de

MRSA media, herkennen wij niet uit onze praktijk. In de richtlijn missen we de getalsmatige onderbouwing van hoe vaak dit het geval is. Voor een advies, waarbij de bewerkelijkheid van de kweek fors toeneemt, is het van belang om te weten hoe groot de bijdrage van de extra inspanning is. Kortom, wij zijn het niet eens met deze aanbeveling zonder onderbouwing.

Response

The use of a growth control is recommended for two reasons: 1) to disapprove MRSA cultures from non-sterile culture sites when the growth control is negative; and 2) as backup for MRSA isolates that are suppressed by the selective agents used in the MRSA screening agar.
The working group agrees with the comment that one should rely on the skills of health professionals to apply appropriate techniques to sample patients. However, the compliance to appropriate specimen collection may be hampered by potential conflicts of interest in case of self-sampling, which is uniformly applied in case of sampling of health care workers. The working group is not aware of any scientific publications on this issue. The first aspect of the recommendation is, thus, based on expert opinion. With respect to the second aspect, there is sufficient evidence to substantiate the recommendation to use the growth control as backup for MRSA isolates that are suppressed by the selective agents used in the MRSA screening agar. Studies evaluating the performance of different chromogenic media have reported variable results. In general, sensitivities tend to vary widely (65% to 89%), both between media and between studies [Luteijn 2011]. In a recent head-to-head comparison of five chromogenic media the sensitivity varied from 81% to 90% [Malhotra 2010].

Changes

No changes.

COMMENT 14

Author

Dr. M.J.H.M. Wolfhagen, *Isala Klinieken (Zwolle)*

Chapter, paragraph, page and line concerned

Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin-resistance / 2.1.3 Laboratory methods / page 13, line 11-12
Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin-resistance / 2.1.3.3 Broth enrichment / page 16, line 11-14

Comment

Ik wilde commentaar geven op het 6,5% NaCl gehalte van de bouillon voor het selectief ophopen van MRSA. Uit eerder onderzoek blijkt dit percentage remmend te werken op de groei van een aantal stammen. Onderzoek, ondermeer op ons lab, laat zien dat voor ons 2,5 % optimaal is [Bruins 2007, Jones 1997].

Response

The working group agrees with the comment that the salt tolerance of MRSA strains varies, and that broths containing >2.5% sodium chloride may be inhibitory for some strains [Bruins 2007, Jones 1997]. Unfortunately, the studies mentioned are *in* vitro studies on selected MRSA strains, and do not provide comparative data on the added value of preincubation of clinical samples in a broth containing 2.5% sodium chloride. Comparative clinical studies have been performed for the use of non-selective or salt only (6.5% sodium chloride) broths, indicating that the sensitivity of chromogenic media is not increased when samples are preincubated in a non-selective broth [Böcher 2010], but is significantly increased when a Mueller-Hinton- or trypticase soy broth supplemented with 6.5% sodium chloride is used (95% and 97% vs. 75% and 64%, respectively) [MacAllister 2011, Verkade 2011]. It can be questioned whether a broth containing 2.5% sodium chloride would increase the sensitivity of chromogenic agar media to the same extent as a broth containing 6.5% sodium chloride, as a sodium chloride concentration of 2.5% may be too low to sufficiently inhibit contaminating flora. In conclusion, currently available data are considered too limited to recommend the use of a broth containing 2.5% sodium chloride. However, a comment on the varying salt tolerance of MRSA strains will be added to paragraph 2.1.3.3.

Changes

- Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin-resistance / 2.1.3 Laboratory methods / page 13, line 11-12: 'Mueller-Hinton broth with 6.5% NaCl OR tryptic soy broth with 6.5% NaCl' is changed into: 'Mueller-Hinton broth supplemented with sodium chloride OR tryptic soy broth supplemented with sodium chloride'
- Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin-resistance / 2.1.3.3 Broth enrichment / page 16, line 14: The following text has been added: '*In vitro* studies have shown that the salt tolerance of MRSA strains may vary, and that a broth containing >2.5% sodium chloride may be inhibitory for some strains [Bruins 2007, Jones 1997]. Unfortunately, comparative data on the added value of preincubation of clinical samples in a broth containing 2.5% sodium chloride are not available. It should be noted that it is currently unclear whether a broth containing 2.5% sodium chloride would increase the sensitivity of chromogenic agar media to the same extent as a broth containing 6.5% sodium chloride. A sodium chloride concentration of 2.5% may be too low to sufficiently inhibit contaminating flora.'

COMMENT 15

Author

Dr. B. Postma, Dr. E.M. Mascini, Drs. T-N. Le, Drs. R.W. Bosboom, Dr. A.A. van Zwet, Dr. A.J. van Griethuysen, Dr. M.A. Schouten, Dr. C.M.A. Swanink, Drs. J. Keijman, *Maatschap Medische Microbiologie & Immunologie Gelderland (Arnhem)*

Chapter, paragraph, page and line concerned

Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin-resistance / 2.1.3.3 Broth enrichment

Comment

De richtlijn gaat uit van de volgende opties:

1. Het afgenomen materiaal direct enten op een chromogene plaat.
2. Het afgenomen materiaal direct in een PCR brengen.
3. Het afgenomen materiaal in een ophopingsbuis brengen en na 18-24 uur afenten op een chromogene plaat (deze wordt dan na 1 en 2 dagen afgelezen).

Opties 1 en 2 worden geschikt geacht voor een snelle voorlopige uitslag, optie 3 voor een definitieve uitslag.

De sensitiviteit van de chromogene plaat en PCR, beiden onder ophoping, zijn vergelijkbaar, zoals ook uit de gegevens in de conceptrichtlijn blijkt. Een toegenomen sensitiviteit van de PCR na ophoping is te verwachten, net als bij de chromogene agar.

Wij stellen voor om een vierde optie toe te voegen, waarbij het afgenomen materiaal na 18-24 uur incubatie in een ophopingsbouillon in een PCR wordt gebracht. Dit is momenteel in veel laboratoria gebruikelijk. Naar onze mening zou een positief PCR resultaat vervolgens worden bevestigd m.b.v. kweek terwijl een negatief PCR resultaat kan worden beschouwd als een definitieve uitslag.

Response

The working group does not agree to include the suggested fourth option as recommended method, i.e. to preincubate clinical samples before the use of molecular detection methods.

It has been suggested that preincubation of clinical samples before applying molecular methods would increase the yield of screening to the same extent as has been shown for chromogenic media. However, to our knowledge no head-to-head comparative data on the added value of broth enrichment prior to molecular detection are currently available. A recent study that compared the performance of molecular methods after preincubation of clinical samples reported a sensitivity of 94% compared with culture results using a selective phenol red mannitol broth [Kerremans 2008]. No comparison with direct molecular detection methods was made in this study. Moreover, a selective method was used as a reference method, which may have resulted in overestimation of the sensitivity. Besides these limitations, it should be noted that the added value of direct molecular detection methods, i.e. the potential to reduce the number of pre-emptive isolation days, disappears when samples are preincubated.

With respect to the remark that a negative PCR result can be considered as a definitive result, it is important to note that the target used for molecular detection, SCC*mec-orfX*, has a relatively high variability that may result in failure to detect some strains, which may vary in time and between regions. It is, therefore, recommended to confirm negative test results by conventional cultures.

Changes

- Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin-resistance / 2.1.3.3 Broth enrichment / page 16, line 16: The following text has been added: 'It has been suggested that preincubation of clinical samples before applying molecular methods would increase the yield of screening to the same extent as has been shown for chromogenic media. However, to our knowledge no head-to-head comparative data on the added value of broth enrichment prior to molecular detection are currently available. A recent study that compared the performance of molecular methods after preincubation of clinical samples reported a sensitivity of 94% compared with culture results using a selective phenol red mannitol broth [Kerremans 2008]. No comparison with direct molecular detection methods was made in this study. Moreover, a selective method was used as a reference method, which may have resulted in overestimation of the sensitivity. Besides these limitations, it should be noted that the added value of direct molecular detection methods, i.e. the potential to reduce the number of pre-emptive isolation days, disappears when samples are preincubated.'
- Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin-resistance / 2.1.3.3 Broth enrichment / page 16, line 18: The following text has been added: 'The working group takes the view that there is insufficient evidence to currently recommend the use of broth enrichment in combination with molecular detection methods.'

COMMENT 16

Author

Dr. G.A. Kampinga, namens artsen-microbioloog UMCG, *UMC Groningen (Groningen)*

Chapter, paragraph, page and line concerned

Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin-resistance / 2.1.3.4 Pooling of samples / page 16, line 20-30

Comment

De tekst over poolen is onduidelijk. Van medewerkers mogen kweken wel gepoold worden. Van patiënten wordt aanbevolen dat kweken niet gepoold worden ingezet. Wij zijn gewoon alle kweken apart af te nemen, maar bij een 1ste screening de neus en keelswab gepoold in te zetten. Wij poolen bewust niet perineum bij neus en keel, omdat enterokokken ook groenig kleuren op de door ons gebruikte agar en we het risico op overgroei voor deze lokalisatie ook groter vinden. Het niet mogen poolen van neus en keel bij patiënten bij een screening werkt kostenverhogend terwijl het risico op overgroei en daardoor missen van een MRSA ons gering lijkt. Bij een 1ste positieve bevinding herhalen we altijd de kweek en dan wordt wel alles apart ingezet. Bij patiënten geldt ook dat er zelden een reden is voor acuut toepassen van MRSA-decontaminatie. Dit is juist wel het geval bij medewerkers, daar mag echter wel weer gepoold worden.

Response

For patients it is recommended to use a separate swab for each culture site, and not to pool samples. First, the site of carriage may impact clinical decisions and treatment strategies [SWAB 2012]. It can be questioned, whether it is acceptable to delay clinical decision making and treatment in patients by the need to repeat cultures in case of MRSA-positive pooled initial samples; the more as patients may be on empirical antimicrobial treatment after the initial sampling. Second, the effect of pooling of clinical samples on the diagnostic performance of conventional culture methods is insufficiently clear. Although reported *S. aureus* carriage rates are comparable for groups of individuals for whom samples are processed either pooled or separate [Mertz 2007], a direct comparison of pooled vs. separate processing of individual samples showed a reduced sensitivity (86%) for pooled processing [Grmek-Kosnik 2005]. For PCR based direct molecular detection methods, pooling of samples has been shown to increase the rate of inhibition and reduce the sensitivity [Jeyratnam 2008B, Kelley 2009, Svent-Kucina 2009, Wassenberg 2011]. Finally, culture orders are becoming increasingly diverse, i.e. orders do not only request for the detection of MRSA, but also for the detection of highly-resistant Enterobacteriaceae (HRE) and/or VRE. The appropriateness to pool samples for the detection of HRE and/or VRE remains to be determined.

In conclusion, based on currently available data the working group does not agree with the suggestion of the authors to pool samples from patients in non-outbreak situations.

The working group agrees with the comment that the arguments against pooling of samples also apply to the pooling of samples of healthcare workers. The decision to pool samples of healthcare workers for cost-saving reasons should, therefore, always be weighed against the disadvantages of pooling mentioned above.

Changes

- Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin-resistance / 2.1.3.4 Pooling of samples / page 16, line 20-30: The text has been replaced by: ‘For patients it is recommended to use a separate swab for each culture site, and not to pool samples First, the site of carriage may impact clinical decisions and treatment strategies [SWAB 2012]. Second, the effect of pooling of clinical samples on the diagnostic performance of conventional culture methods is insufficiently clear. Although reported *S. aureus* carriage rates are comparable for groups of individuals for whom samples are processed either pooled or separate [Mertz 2007], a direct comparison of pooled vs. separate processing of individual samples showed a reduced sensitivity (86%) for pooled processing [Grmek-Kosnik 2005]. For PCR based direct molecular detection methods, pooling of samples has been shown to increase the rate of inhibition and reduce the sensitivity [Jeyratnam 2008B, Kelley 2009, Svent-Kucina 2009, Wassenberg 2011]. Another aspect that should be taken into account is that pooling of samples limits the use of a growth control (see 2.1.3.2). Pooling of patient samples can be considered in case of an outbreak. For screening of healthcare workers it can be considered to use one swab to sample all three culture sites (nose, throat, and perineum), or to pool swabs. However, once MRSA carriage is detected all sites should be sampled separately before treatment is initiated, as the site of carriage determines the treatment strategy [SWAB

2012]. The decision to pool samples for cost-saving reasons should, therefore, always be weighed against the disadvantages of pooling.'

COMMENT 17

Author

Dr. B. Postma, Dr. E.M. Mascini, Drs. T-N. Le, Drs. R.W. Bosboom, Dr. A.A. van Zwet, Dr. A.J. van Griethuysen, Dr. M.A. Schouten, Dr. C.M.A. Swanink, Drs. J. Keijman, *Maatschap Medische Microbiologie & Immunologie Gelderland (Arnhem)*

Chapter, paragraph, page and line concerned

Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin-resistance / 2.1.5.2 Patient information system

Comment

De in deze conceptrichtlijn gesuggereerde systematiek van een voorlopige (snelle) uitslag en een definitieve uitslag (na 3 dagen) sluit niet aan op de werkwijze in de WIP richtlijn MRSA. Wij concluderen uit de conceptrichtlijn dat opheffen van de isolatiemaatregelen bij een negatieve voorlopige uitslag als mogelijkheid wordt opengelaten, de WIP richtlijn zou dit dan ook zo moeten vermelden.

Response

This comment relates to the indications for (discontinuation of) isolation of patients with MRSA, which is beyond the scope of this guideline on the detection of antimicrobial resistance. The author of the comment is referred to the Dutch Workingparty on Infection Prevention that has developed the guideline 'MRSA hospital' [WIP 2007].

Changes

No changes.

COMMENT 18

Author

Dr. G.A. Kampinga, namens artsen-microbioloog UMCG, *UMC Groningen (Groningen)*

Chapter, paragraph, page and line concerned

Chapter 5 – Enterobacteriaceae / 5.1 Extended-spectrum beta-lactamases

Comment

De BMRO WIP richtlijn stelt dat screening op BMRO aanbevolen wordt bij overname uit een buitenlandsziekenhuis indien de patient wordt opgenomen op een hoog risico afdeling, zoals een IC. De vraag rijst of het niet logischer is in het algemeen te stellen dat screening op bijzondere resistente gram-negatieve staven (BRGNS) (en VRE) wenselijk is bij patiënten die in het buitenland ziekenhuis

hebben gelegen en worden opgenomen in een ziekenhuis, net zoals nu ook voor MRSA wordt gesteld. Met name uitbraken van Klebsiella's zijn in ziekenhuizen niet beperkt tot IC-patiënten. Thans is er verschil in beleid tussen ziekenhuizen binnen een zelfde regio wat bij overname van patiënten tot verwarring kan leiden.

Response

This comment relates to the indications for screening for HRMO, which is beyond the scope of this guideline on the detection of antimicrobial resistance. The author of the comment is referred to the Dutch Workingparty on Infection Prevention that has developed the guideline 'Measures to prevent transmission of highly resistant microorganisms' that includes recommendations on the indications for screening for HRMO' [WIP 2005].

Changes

No changes.

CHANGES MADE ON BEHALF OF MEMBERS OF THE WORKING GROUP

The following changes have been made on behalf of members of the working group:

- Title page / page 1, line 25: 'Dr. M.C. Vos' has been changed into 'Prof. dr. M.C. Vos'.
- Table of contents / page 4, line 5: 'Appendix E – Revisions' has been added.
- Chapter 1 – General introduction / 1.5 Working group / 1.5.1 Working group members / page 6, line 26: 'Dr. M.C. Vos' has been changed into 'Prof. dr. M.C. Vos'.
- Chapter 1 – General introduction / 1.5 Working group / 1.5.2 Conflict of interest / page 7, line 3: 'Dr. M.C. Vos' has been changed into 'Prof. dr. M.C. Vos'.
- Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin resistance / page 10, line 5: 'Dr. M.C. Vos' has been changed into 'Prof. dr. M.C. Vos'.
- Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin resistance / 2.1.2 Detection of carriage / page 12, line 9: 'working day' has been changed into 'working shift'.
- Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin resistance / page 16, line 29: Reference Kerremans 2008 has been deleted (reference does not pertain to direct molecular detection methods).
- Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin resistance / 2.1.4 Contact tracing / page 18, line 21: The following sentence has been added: 'Dependent on the diagnostic characteristics of the 'known' strain, the methods used may be adjusted in order to improve the efficiency of MRSA detection.'
- Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin resistance / 2.1.7 Recommendations / page 20, line 14: 'working day' has been changed into 'working shift'.

COMMENTS THAT ARE NOT APPLICABLE TO THE REVISION OF VERSION 1

Concept version 1.1 of the guideline was open for comments on:

- Chapter 2.1 *Staphylococcus aureus* – methicillin resistance; and
- The addition of the quality indicator 'laboratory turnaround time' to
    - Chapter 1 – General introduction
    - Chapter 5.1 Enterobacteriaceae – extended-spectrum beta-lactamases
    - Chapter 5.2 Enterobacteriaceae – carbapenemases.

Comments that relate to parts of the guideline that have been adopted in 2011 and are currently not open for comment will be published in version 2, but will not be dealt with until the next revision.

COMMENT 19

Author

Dhr. E. Heddema, *Orbis Medisch Centrum (Sittard-Geleen)*

Chapter, paragraph, page and line concerned

Chapter 5 – Enterobacteriaceae / 5.1 Extended-spectrum beta-lactamases / 5.1.2.2 Number of cultures / page 24, line 11-14

Comment

De richtlijn stelt dat: 'Therefore, the working group has decided to follow the current recommendation for the detection of carriage of *Salmonella* spp. [Behravesh 2008], i.e. patients can be considered to be no longer carrying HRE if two culture sets, collected at least 24 hours apart, and at least 48 hours after discontinuation of antibiotic therapy are negative.'

Over aankweek wordt gezegd: 'At this point the working group takes the view that there is insufficient evidence to provide a firm recommendation on the use of broth enrichment for the detection of ESBL-E.'

Dit is volgens mij in tegenspraak omdat bij Salmonella dragerschapskweken vrijwel standaard aankweek gebruikt wordt om de detectie te verhogen. Wanneer dus literatuur betreffend Salmonella als uitgangspunt word genomen waarin aankweek een duidelijke rol heeft, moet je dit ook doortrekken naar de BRMO kweek en aankweek adviseren indien men na 2 kweken iemand als negatief wil beschouwen. Of anderszins een oplossing zoeken.

Response

This comments relates to a part of the guideline that has been adopted in 2011, and is currently not open for comment. The comment will be dealt with in the next revision.

COMMENT 20

Author

Dr. G.A. Kampinga, namens artsen-microbioloog UMCG, *UMC Groningen (Groningen)*

Chapter, paragraph, page and line concerned

Chapter 5 – Enterobacteriaceae / 5.1 Extended-spectrum beta-lactamases / 5.1.2.1 Culture sites / page 24 / line 2

Comment

Is er evidence dat het kweken van wonden meer dragers op BRGNS op levert?

Response

This comments relates to a part of the guideline that has been adopted in 2011, and is currently not open for comment. The comment will be dealt with in the next revision.

COMMENT 21

Author

Dr. G.A. Kampinga, namens artsen-microbioloog UMCG, *UMC Groningen (Groningen)*

Chapter, paragraph, page and line concerned

Chapter 5 – Enterobacteriaceae / 5.1 Extended-spectrum beta-lactamases / 5.1.3.5 Susceptibility testing / page 30 / line 11-16

Comment

Is er informatie beschikbaar wat de opbrengst is van het standaard testen op ESBL mbv cefepim bij Enterobacters en Citrobacters die een MIC van >1 voor cefotaxim of ceftazidim hebben, een MIC van <=1 voor cefepim in de Vitek en waarbij verder geen aanwijzing is voor andere verworven resistenties zoals resistentie tegen aminoglycosiden of co-trimoxazol? Als deze isolaten standaard getest moeten worden betekent dat een behoorlijke toename in ESBL testen, terwijl de a priori kans wellicht heel laag is. In het artikel van Cohen Stuart uit 2011 zie ik helaas geen aparte analyse van de sensitiviteit van de Vitek als ook rekening wordt gehouden met co-resistenties. Hier speelt ook een kosten-baten analyse een rol.

Response

This comments relates to a part of the guideline that has been adopted in 2011, and is currently not open for comment. The comment will be dealt with in the next revision.

COMMENT 22

Author

Dr. G.A. Kampinga, namens artsen-microbioloog UMCG, *UMC Groningen (Groningen)*

Chapter, paragraph, page and line concerned

Chapter 5 – Enterobacteriaceae / 5.1 Extended-spectrum beta-lactamases / 5.1.5.2 Patient information system / page 31 / line 18-27

Comment

Er wordt zeer stellig gezegd dat alleen een carbapenem als S moet worden uitgeslagen. We zijn gewend amoxicilline-clavulaanzuur standaard als R uit te slaan (mede omdat amoxicilline voor Enterobacteriaceae al niet zo'n optimaal middel is en het middel ook in lage orale dosis wordt toegepast) en piperacilline-tazobactam (PITA) uit te slaan zoals is gemeten. Indien PITA S is , wordt altijd een opmerking toegevoegd dat dit middel alleen mag worden toegepast in overleg met de arts-microbioloog. Het behandelen van niet gecompliceerde urineweginfecties standaard met een carbapenem , heeft als nadeel dat er een toename gaat plaats vinden in gebruik van carbapenems. Resistentie ontwikkeling onder therapie met een carbapenem hebben we al meerdere keren gezien. Het sparen van een carbapenem voor ernstige infecties lijkt ons daarom gerechtvaardigd. We zouden het logischer vinden te zeggen dat er altijd een waarschuwing moet worden vermeld en niet algemeen te stellen dat geen enkele penicilline als S mag worden uitgeslagen.

Response

This comments relates to a part of the guideline that has been adopted in 2011, and is currently not open for comment. The comment will be dealt with in the next revision.

APPENDIX E – REVISIONS

VERSION 1.0 (adopted 2011.11.17)

New chapters

- Chapter 1 – General introduction
- Chapter 5 – Enterobacteriaceae / 5.1 Extended-spectrum beta-lactamases
- Chapter 5 – Enterobacteriaceae / 5.2 Carbapenemases

VERSION 2.0 (adopted 2012.11.15)

New chapters

- Chapter 2 – *Staphylococcus aureus* / 2.1 Methicillin resistance
- Appendix E – Revisions

Revisions

- Chapter 1 – General introduction: addition of paragraph 1.6.4 Quality indicators
- Chapter 5 – Enterobacteriaceae / 5.1 Extended-spectrum beta-lactamases: addition of paragraph 5.1.6 Quality indicators
- Chapter 5 – Enterobacteriaceae / 5.2 Carbapenemases: addition of paragraph 5.2.6 Quality indicators