Journal of Global Antimicrobial Resistance 2 (2014) 155–161

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Antimicrobial susceptibility among Gram-positive and Gram-negative isolates collected in Europe between 2004 and 2010

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ARTICLE INFO

Article history:
Received 20 March 2013
Received in revised form 19 March 2014
Accepted 6 May 2014

Keywords:
Tigecycline Evaluation and Surveillance Trial
Antimicrobial susceptibility
Europe

ABSTRACT

Here we report on the in vitro activity of a suite of antimicrobial agents against Gram-negative and Gram-positive pathogens collected in Europe between 2004 and 2010 as part of the Tigecycline Evaluation and Surveillance Trial (T.E.S.T.). Clinical and Laboratory Standards Institute (CLSI) broth microdilution methodologies were used to determine minimum inhibitory concentrations. CLSI interpretive criteria were applied for all antimicrobial agents to establish susceptibility; European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints were used for tigecycline. In total, 46,921 Gram-negative and 19,174 Gram-positive isolates were included in this study. Extended-spectrum β-lactamases increased in proportion from 15.7% to 21.1% among *Klebsiella pneumoniae* and from 9.7% to 16.1% among *Escherichia coli* isolates between 2004–2007 and 2010. *E. coli* susceptibility decreased to most antimicrobials but it remained highly susceptible (>98%) to tigecycline and meropenem. *Acinetobacter baumannii* susceptibility also decreased to most agents. The proportion of meticillin-resistant *Staphylococcus aureus* (MRSA) decreased from 25.7% to 19.4% over the study period. Antimicrobial susceptibility has decreased among many of the pathogens observed in the T.E.S.T. surveillance study between 2004–2007 and 2010.

1. Introduction

An alarming escalation in antimicrobial resistance has been seen in Europe in recent decades both among Gram-positive and Gram-negative pathogens. However, the prevalence of many resistant Gram-positive pathogens has become relatively stable in recent years, although regional fluctuations occur due to localised outbreaks of resistant phenotypes [1]. Also, the prevalence of meticillin-resistant *Staphylococcus aureus* (MRSA) in several countries has stabilised or even begun to decrease in recent years [2]. Gram-negative pathogens are more concerning, with dramatic increases in multidrug resistance reported among several organisms, including *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [2,3].

Carbapenems have typically been the first drug of choice in recent years in Europe for empirical therapy against infections caused by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae such as *K. pneumoniae* [4]. This extensive use of carbapenems has resulted in the development of resistance mechanisms among Enterobacteriaceae that are active against carbapenems as well as many β-lactams [5]. The dissemination of multidrug-resistant, carbapenemase-producing pathogens and the transfer of plasmids conveying the genes responsible for this multidrug resistance are of major concern in Europe [6].

In this study, the in vitro activity of tigecycline [7] was assessed against a range of clinically important bacterial pathogens in the Tigecycline Evaluation and Surveillance Trial (T.E.S.T.), an ongoing comparative global surveillance study. Nørskov-Lauritsen reported T.E.S.T. data on Gram-positive (N = 7988) and Gram-negative (N = 16,760) aerobic isolates collected in Europe from the start of the study in 2004–2007 [8]. In the current paper, we update the results of Nørskov-Lauritsen et al. [8] by examining all aerobes collected in Europe as a part of T.E.S.T. between 2004 and 2010.

2. Materials and methods

Collection of clinical isolates for T.E.S.T. began at study centres globally in 2004 and is ongoing. Study centres were instructed to

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http://dx.doi.org/10.1016/j.jgar.2014.05.001
2213-7165/

identify and submit in each year consecutive and clinically significant (probable causative agents of infection as determined by local criteria), non-duplicate isolates to a maximum of 25 for each of *S. aureus*, *E. coli*, *Enterobacter* spp. and *Klebsiella* spp.; 20 for *P. aeruginosa*; 15 for each of *Streptococcus pneumoniae*, *Enterococcus* spp., *Haemophilus influenzae* and *Acinetobacter* spp.; and 10 for each of *Streptococcus agalactiae* and *Serratia* spp. All body sites were considered acceptable isolate sources, although a maximum of 25% of isolates could be derived from urine. Only a single isolate was accepted per patient; isolate inclusion was independent of the medical history, previous antimicrobial use, sex or age of a patient. No banked or stored isolates were accepted into this study.

Each study centre determined the local minimum inhibitory concentrations (MICs) of a defined panel of antimicrobial agents using broth microdilution methodology [Sensititre® plates (TREK Diagnostic Systems, West Sussex, England) or MicroScan® panels (Siemens, Sacramento, CA, USA)], according to the guidelines published by the Clinical and Laboratory Standards Institute (CLSI) [9]. The panel included amoxicillin/clavulanic acid, ampicillin, ceftriaxone, imipenem, levofloxacin, meropenem, minocycline, piperacillin/tazobactam (TZP) and tigecycline. In addition, Gram-positive isolates were tested against linezolid, penicillin and vancomycin (the panel for *S. pneumoniae* also included azithromycin, clarithromycin, clindamycin and erythromycin), whilst amikacin, cefepime and ceftazidime were included in the panel for Gram-negative isolates; meropenem testing using Sensititre commenced in 2006. Quality control (QC) strains used were *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 25922 *and P. aeruginosa* ATCC 27853. QC testing was carried out on each day of MIC testing under identical conditions as test isolates; test results were accepted only when QC testing results were within ATCC expected ranges.

To determine antimicrobial susceptibility, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints were used for tigecycline [10]. Otherwise, CLSI interpretive criteria were applied [11]; carbapenem breakpoints of Enterobacteriaceae were revised in 2010 [12]. For *S. pneumoniae*, MIC testing was carried out using non-meningitis breakpoints [11].

Isolates of *E. coli* and *Klebsiella* spp. were tested locally for ESBL production according to CLSI guidelines [9] using Mueller–Hinton agar (Remel, Inc., Lenexa, KS) and disks with cefotaxime (30 μg), cefotaxime/clavulanic acid (30/10 μg), ceftazidime (30 μg) and ceftazidime/clavulanic acid (30/10 μg) (Oxoid, Inc., Ogdensburg, NY). QC strains used were ESBL-positive *K. pneumoniae* ATCC 700603 and ESBL-negative *E. coli* ATCC 25922. *S. aureus*, *S. pneumoniae*, *H. influenzae* and enterococci were analysed by contributing centres using locally preferred methodologies to identify isolates that were methicillin-resistant, penicillin-resistant, β-lactamase-positive and vancomycin-resistant, respectively.

A central laboratory, Laboratories International for Microbiology Studies [a division of International Health Management Associates, Inc. (IHMA), Schaumburg, IL], confirmed all isolate identifications and managed a centralised database; IHMA also randomly selected 10–15% of isolates annually for QC checks. All isolates were shipped to IHMA using next-day courier using only transport tubes [A.C.T.® II Sterile Pack Tubes (Remel, Thermo Fisher, Lenexa, KS) for *S. pneumoniae* and chocolate agar transport tubes (Remel, Thermo Fisher) for all other organisms]. IHMA collates all isolate data in an Excel spreadsheet (Microsoft Corp., Redmond, WA), which is provided to Micron Research (Ely, UK) who then carry out data analyses using SAS statistical software v.8.2 (SAS Institute Inc., Cary, NC).

This report includes all isolates submitted as a part of T.E.S.T. in Europe between 2004 and 2010. Data for 2004–2007 do not match those of Nørskov-Lauritsen et al. [8] owing to the temporary quarantining of some isolates from the T.E.S.T. 2012 database (due to MIC re-testing requests) or because some isolates were non-evaluable and were thus permanently removed from the T.E.S.T. database (e.g. cultures that did not survive shipping from collecting centre to IHMA, were never received by IHMA or could not be resuscitated after storage). Results are not given in this report for antimicrobials against which an organism is considered to be intrinsically resistant.

3. Results

A total of 46,921 Gram-negative and 19,174 Gram-positive isolates were collected at European centres during T.E.S.T. in 2004–2010. Twenty-six countries participated (Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Norway, Poland, Portugal, Romania, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, The Netherlands and the UK), but over one-half of the isolates originated from France, Germany, Italy or Spain.

3.1. Gram-negative pathogens

3.1.1. A. baumannii

Minocycline susceptibility decreased from 94.1% in 2004–2007 to 78.8% in 2010 (Table 1). Susceptibility was lower among all remaining agents, ranging from 72.4% for amikacin in 2004–2007 to 19.4% for ceftriaxone in 2010. The lowest MIC_{90} values (MIC for 90% of the isolates) were reported for tigecycline (1 mg/L in 2004–2007, 2 mg/L in 2008–2010; data not shown). Meropenem resistance ($n = 1255$) did not have any effect on the activity of minocycline (73.7% susceptible) or tigecycline ($MIC_{90} = 2$ mg/L) (data not shown).

3.1.2. Enterobacter *spp.*

The highest susceptibility rates among *Enterobacter* spp. were associated with amikacin (≥96.2%) and meropenem (≥95.0%). MIC_{90} values remained at ≤2 mg/L for tigecycline and meropenem. Minocycline susceptibility decreased from 79.2% in 2004–2007 to 52.0% in 2010 (Table 1). Meropenem resistance was observed among 149 *Enterobacter* isolates; the most active agents against these isolates were amikacin (70.5% susceptible) and tigecycline (65.1% susceptible) (data not shown).

3.1.3. E. coli

Susceptibility fell by 5–10 percentage points to most antimicrobials during this study; however, *E. coli* remained highly susceptible to meropenem (≥99.0%), tigecycline (≥98.4%) and amikacin (≥97.9%) (Table 1). In total, 30 isolates were resistant to meropenem (8 in Italy, 4 in Romania, 3 each in Germany and Spain, 2 each in Denmark, Greece and Slovenia and 1 each in Belgium, Bulgaria, Croatia, France, Ireland and Poland); 24 of these were collected in 2008–2009. Only tigecycline showed good activity against these meropenem-resistant isolates, with 93.3% tigecycline susceptible (data not shown). The rate of ESBL-producing *E. coli* was 9.7% across Europe during 2004–2007 but increased to 16.1% in 2010 (data not shown). The highest rates of ESBL-producing *E. coli* were recorded in Portugal (25.7%), Bulgaria (24.7%), Italy (24.2%), Greece (20.6%) and Austria (20.4%) (Table 2). Among ESBL-producers, decreasing susceptibility was observed to the β-lactams (e.g. TZP, 78.4% in 2004–2007, 68.9% in 2010), levofloxacin (26.2% in 2004–2007, 15.8% in 2010) and minocycline (62.7% in 2004–2007, 56.3% in 2010) (data not shown).

3.1.4. H. influenzae

H. influenzae remained highly susceptible (≥97.3%; $MIC_{90} \leq 1$ mg/L) to all antimicrobials over the total duration of