Diagnostic Microbiology and Infectious Disease xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

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

Antimicrobial susceptibility and extended-spectrum beta-lactamase rates in aerobic gram-negative bacteria causing intra-abdominal infections in Vietnam: report from the Study for Monitoring Antimicrobial Resistance Trends (SMART 2009–2011)

Douglas J. Biedenbach [a,*], Samuel K. Bouchillon [a], Daryl J. Hoban [a], Meredith Hackel [a], Doan Mai Phuong [b], Tran Thi Thanh Nga [c], Nguyen Tran My Phuong [d], Tran Thi Lan Phuong [e], Robert E. Badal [a]

[a] International Health Management Associates, Inc., Schaumburg, IL, USA
[b] Bach Mai Hospital, Hanoi, Vietnam
[c] Cho Ray Hospital, Ho Chi Minh City, Vietnam
[d] Binh Dan Hospital, Ho Chi Minh City, Vietnam
[e] Viet Duc Hospital, Hanoi, Vietnam

ARTICLE INFO

Article history:
Received 13 December 2013
Received in revised form 9 May 2014
Accepted 10 May 2014
Available online xxxx

Keywords:
ESBL
Vietnam
Resistance
SMART study

ABSTRACT

Treatment options for multidrug-resistant pathogens remain problematic in many regions and individual countries, warranting ongoing surveillance and analysis. Limited antimicrobial susceptibility information is available for pathogens from Vietnam. This study determined the bacterial susceptibility of aerobic gram-negative pathogens of intra-abdominal infections among patients in Vietnam during 2009–2011. A total of 905 isolates were collected from 4 medical centers in this investigation as part of the Study for Monitoring Antimicrobial Resistance Trends. Antimicrobial susceptibility and extended-spectrum beta-lactamase (ESBL) rates among the appropriate species were determined by a central laboratory using Clinical and Laboratory Standards Institute methods. Among the species collected, *Escherichia coli* (48.1% ESBL-positive) and *Klebsiella pneumoniae* (39.5% ESBL-positive) represented the majority (46.4%) of the isolates submitted for this study. Ertapenem MIC_{90} values were lowest for these 2 species at 0.12 and 0.25 μg/mL and remained unchanged for ESBL-positive isolates. Imipenem MIC_{90} values were also the same for all isolates and ESBL-positive strains at 0.25 and 0.5 μg/mL, respectively. Ertapenem MIC_{90} values for additional species with sufficient numbers for analysis, including *Enterobacter cloacae*, *Proteus mirabilis*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, were 1, 0.06, >4, and >4 μg/mL, respectively. Analysis of beta-lactamases in a subset of 132 phenotypically ESBL-positive Enterobacteriaceae demonstrated that CTX-M variants, particularly CTX-M-27 and CTX-M-15, were the predominant enzymes. High resistance rates in Vietnam hospitals dictate continuous monitoring as antimicrobial inactivating enzymes continue to spread throughout Asia and globally.

1. Introduction

Intra-abdominal infections (IAIs) are a substantial cause of morbidity and mortality and must be managed and treated with great care as etiological species can vary dramatically, depending upon the organ systems involved (Solomkin et al., 2010a, 2010b). The incidence of post-operative infection also varies and is associated with the invasiveness of the surgical procedure and other factors such as patient age and disease status (Solomkin et al., 2010b). The inappropriate usage of cephalosporins as initial prophylactic therapeutic agents in surgical wards and intensive care units as well as sub-therapeutic dosing for the treatment of IAIs is likely causes of the high extended-spectrum beta-lactamase (ESBL) rates observed in some countries, including Vietnam. Non-prescribed "over the counter" antimicrobial agents that are available in Vietnam and non-compliance by patients are also culpable for the resistance rates observed in this country, including community-associated infections. Referral of patients from other hospitals with infection control problems to these larger hospitals for treatment may complicate the severity of antimicrobial resistance, including ESBL rates that are being observed in these institutions.

It is necessary to have appropriate therapeutic agents and treatment guidelines for antimicrobial intervention that can be used prior to surgery to prevent complicated IAIs (cIAIs) or provide a post-operative therapeutic option if an infection occurs (Alexander et al., 2011; Mazuski et al., 2002a, 2002b). Consensus on the appropriate antimicrobial agents for use in IAI in Asia was provided in 2007, with ertapenem and other carbapenems recommended for use depending upon the severity of the infection (Hsueh and Hawkey, 2007). Several antimicrobial agents are recommended for the empiric treatment of

* Corresponding author. Tel.: +1-847-303-5003; fax: +1-847-303-5601.
E-mail address: dbiedenbach@ihmainc.com (D.J. Biedenbach).

http://dx.doi.org/10.1016/j.diagmicrobio.2014.05.009
0732-8893/

cIAIs until susceptibility profiles of the pathogens are known (Alexander et al., 2011; Hsueh and Hawkey, 2007; Lau et al., 2012; Mazuski et al., 2002a, 2002b; Solomkin et al., 2010a, 2010b). These agents include fluoroquinolones and advanced generation cephalosporins, piperacillin-tazobactam, or carbapenems in several countries, with combination therapy often used to cover a broad range of bacterial species, including both aerobic and anaerobic pathogens. However, countries with high rates of resistance to these classes of agents have a more complicated standard of care dilemma for the use of empiric therapy guidelines and require close surveillance of antimicrobial profiling from a regional and global perspective (Chen et al., 2009, 2011).

Vietnam and other countries in Asia have experienced significantly different resistance rates to some antimicrobial classes. For example, fluoroquinolones are not recommended for use in China, India, Thailand, and Vietnam for the treatment of IAIs; however, this class is considered appropriate first-line therapy in Taiwan (Lau et al., 2012). The use of cephalosporin therapy, including third- and fourth-generation agents, for pathogens commonly associated with IAIs is becoming compromised as well (Turner, 2005), with ESBL-producing strains becoming endemic in several populous countries such as China and India (Livermore, 2012). Although an earlier report indicated that Vietnamese extended-spectrum beta-lactamase-type ESBL enzymes were commonly isolated from Enterobacteriaceae species in some countries in Asia, including Vietnam, more recently, CTX-M family enzymes have become more prominent in many countries in the region such as India and China (Chanawong et al., 2001, 2002; Hawkey, 2008, Yu et al., 2006). Additionally, carbapenemases such as NDM-1 have been detected in isolates of *Escherichia coli* and *Klebsiella pneumoniae* from Vietnam (Hoang et al., 2013). These enzymes will most likely become more widely disseminated due to genetic transfer among species and international travel, and the impact on patient care of the global spread of these resistant isolates is very concerning.

Ertapenem is a carbapenem class agent that is considered to be effective against several types of moderate-to-severe infections, including IAIs, due to the broad coverage that it and other carbapenems have against pathogens from this type of infection, including multidrug-resistant strains such as ESBL producers (Chen et al., 2009, 2011). The focus of this study was to determine the resistance rates to ertapenem and comparator agents among *E. coli* and *K. pneumoniae*, including ESBL-producing isolates, from Vietnam with a more limited emphasis on other less commonly isolated species from IAIs.

2. Materials and methods

A total of 905 isolates were collected from 4 major tertiary referral hospitals in Vietnam during 2009–2011 from patients with IAIs. Sites were located in the 2 largest cities in Vietnam; Ho Chi Minh City (2 sites) and Hanoi (2 sites). Three of the sites contributed isolates all 3 years of this study, while 1 site only contributed isolates in 2010 and 2011. Isolates were collected from multiple hospital wards with the majority obtained from surgical intensive care units. All were from patients with IAIs, including the following infection/organ sources; abscess (15.7%), appendix (34.7%), small intestines/colon/stomach 11.8%, gall bladder/liver (15.4%), peritoneal fluid (15.3%), and other sources. The majority of the pathogens consisted of *E. coli* (697) and *K. pneumoniae* (129), with more limited numbers of *Enterobacter cloacae* (29), *Proteus mirabilis* (19), *Acinetobacter baumannii* (18), and *Pseudomonas aeruginosa* (13). Twelve additional species were isolated in this study, but with less than 10 strains and were therefore excluded from this analysis.

MICs were determined by the Clinical and Laboratory Standards Institute (CLSI) recommended broth microdilution testing method using custom dehydrated MicroScan panels (Siemens Medical Solutions Diagnostics, West Sacramento, CA, USA) tested at a central laboratory in the United States (International Health Management Associates, Inc., Schaumburg, IL, USA) (CLSI M07-A9, 2012a, 2012b). All antimicrobial agents were supplied by the panel manufacturer.

MIC interpretive criteria followed CLSI published guidelines (CLSI M100-S12, 2012a, 2012b). The following antimicrobial agents were included on the panels: amikacin, ampicillin-sulbactam, cefepime, cefotaxime, cefoxitin, ceftazidime, ceftriaxone, ciprofloxacin, levofloxacin, ertapenem, imipenem, and piperacillin-tazobactam. These represent commonly recommended or used antimicrobial agents for the treatment of IAI, depending upon the country or region.

Quality control (QC) testing was performed on each day of testing using ATCC control strains *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *E. coli* ATCC 35218 following CLSI and manufacturer guidelines (CLSI, M100-S12, 2012a and 2012b). Results were included in the analysis only when corresponding QC results were within the acceptable ranges.

E. coli, *K. pneumoniae*, and *P. mirabilis* were tested for ESBL production according to CLSI guidelines (CLSI, M100-S12, 2012a and 2012b). An organism was categorized as ESBL-positive if there was at least an 8-fold decrease in the MIC of cefotaxime or ceftazidime combined with clavulanic acid compared to the MIC of either drug alone.

A subset of 132 phenotypically ESBL-positive or ertapenem non-susceptible (MIC >0.5 μg/mL) Enterobacteriaceae was randomly selected and was from all 4 hospitals. Isolates were molecularly characterized to determine the presence of beta-lactamase genes as follows: DNA was extracted using the QIAamp DNA Mini Kit and the QIAcube instrument (Qiagen, Valencia, CA, USA). Microarray assays were performed using the Check-MDR CT101 test (Check-Points Health BV, Wageningen, the Netherlands) according to manufacturer's instructions to identify TEM-, SHV-, and CTX-M-type ESBLs, AmpC genes (CMY, DHA, FOX, MOX, ACC, MIR and ACT), and KPC and NDM-type carbapenemases. PCR for characterization of ESBLs was performed in an ABI9700 thermocycler (Applied Biosystems, Carlsbad, CA, USA). *bla* genes of the TEM, SHV, CTX-M, AmpC, KPC, and NDM types were amplified as previously described (Lascols et al., 2011; Mulvey et al., 2004; Nuesch-Inderbinen et al., 1996; Perez-Perez and Hanson, 2002; Speldooren et al., 1998; Yigit et al., 2001). Multiplex amplification was performed to detect bla_{VIM} and $bla_{\text{OXA-48}}$ genes using primers previously described (Dallenne et al., 2010).

PCR was carried out with the Fast Cycling PCR Kit (Qiagen). Purification of the PCR products was performed using Exo-SAP-IT® (USB, Cleveland, OH, USA). PCR amplified products were sequenced using the ABI 3730XL DNA analyzer (Applied Biosystems). Nucleotide sequences were analyzed with SeqScape v. 7.0 (Applied Biosystems) and compared to sequences available on the Internet at the National Center for Biotechnology Information Web site (www.ncbi.nlm.nih.gov). Statistical analyses were performed by Fisher's exact test, 2 tailed, using GraphPad Quick Calcs (2005; GraphPad Software, Inc., San Diego, CA, USA). Any $P < 0.01$ was considered statistically significant.

3. Results

E. coli was the most frequently collected pathogen in this study, with 697 isolates in this collection. An ESBL phenotype was observed for 335 isolates, which was 48.1% in this collection (Tables 1 and 2). Ertapenem had the lowest MIC_{90} value (0.12 μg/mL) against this species, followed by imipenem (0.25 μg/mL). Susceptibility rates for *E. coli* ranged from 25.5% for ampicillin-sulbactam to 99.4% for imipenem. Only imipenem, ertapenem, and amikacin had susceptibility percentages ≥97%, while piperacillin-tazobactam retained activity against over 90% of the isolates collected in this study. Ampicillin-sulbactam was inactive against the vast majority of the *E. coli* isolates. Approximately 50% of Vietnamese *E. coli* isolates were non-susceptible to ciprofloxacin and levofloxacin. The second most commonly isolated IAI pathogen collected in this study was *K. pneumoniae* which, similarly to the *E. coli* isolates, had high rates of resistance to the cephalosporins due to a 39.5% ESBL rate (Tables 1 and 2). Ertapenem had the lowest MIC_{90} value (0.25 μg/mL) against *K. pneumoniae* followed by imipenem (MIC_{90}, 0.5 μg/mL). Susceptibility to piperacillin-tazobactam was 82.9%, substantially higher than ampicillin/

Please cite this article as: Biedenbach DJ, et al, Antimicrobial susceptibility and extended-spectrum beta-lactamase rates in aerobic gram-negative bacteria causing intra-abdominal ..., Diagn Microbiol Infect Dis (2014), http://dx.doi.org/10.1016/j.diagmicrobio.2014.05.009