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Antimicrobial Susceptibility Studies

Characterization of extended-spectrum beta-lactamases and antimicrobial resistance of *Klebsiella pneumoniae* in intra-abdominal infection isolates in Latin America, 2008–2012. Results of the Study for Monitoring Antimicrobial Resistance Trends

Krystyna M. Kazmierczak *, Sibylle H. Lob, Daryl J. Hoban, Meredith A. Hackel, Robert E. Badal, Samuel K. Bouchillon

International Health Management Associates Inc., Schaumburg, IL, USA

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ABSTRACT

The Study for Monitoring Antimicrobial Resistance Trends has monitored the in vitro activity of several recommended antimicrobials used in the management of intra-abdominal infections (IAIs) globally since 2002. In this report, we document the changing susceptibility patterns to recommended antimicrobials in *Klebsiella pneumoniae* isolates from patients with IAIs in 11 Latin American countries between 2008 and 2012 and describe the beta-lactamases encoded by phenotypically extended-spectrum beta-lactamase (ESBL)–positive and ertapenem-nonsusceptible isolates. Overall, the incidence of phenotypically ESBL-positive *K. pneumoniae* did not change significantly from 2008 (40.4%) to 2012 (41.2%) ($P > 0.05$). However, trend analysis documented an increase in isolates encoding *K. pneumoniae* carbapenemase (KPC) or both KPC and an ESBL. Decreasing susceptibility ($P < 0.05$) was noted for cefepime, ceftazidime, ceftriaxone, ertapenem, and imipenem among all *K. pneumoniae*, as well as for cefepime, cefotaxime, cefoxitin, ceftriaxone, ertapenem, and imipenem among ESBL-positive isolates, while susceptibility of ESBL-negative isolates to ampicillin-sulbactam actually increased ($P < 0.05$).

1. Introduction

Klebsiella pneumoniae is perhaps the second most common gram-negative aerobic pathogen found in patients with complicated intra-abdominal infections (IAIs) worldwide (Hawser et al., 2011; Hoban et al., 2010). This pathogen has demonstrated unique abilities to acquire and disseminate a variety of extended-spectrum beta-lactamases (ESBLs) including enzymes of the TEM, SHV, and CTX-M families, as well as AmpC cephalosporinases, and increasingly carbapenemases (Cuzon et al., 2010; Munoz-Price et al., 2013). Treatment options for complicated IAIs must consider the epidemiology of pathogens associated with IAI including multidrug-resistant *Escherichia coli*, *K. pneumoniae*, and *Acinetobacter* spp. Resistance patterns have been examined and reported from many geographical regions globally over the past 10 years. However, limited data are available on both resistance patterns and the diversity and evolution of ESBLs in *K. pneumoniae* from Latin American countries (Fernandez-Canigia and Dowzicky, 2012; Gales et al., 2012; Guzman-Blanco et al., 2014; Jones et al., 2013; Minarini et al., 2007; Reinert et al., 2007; Rossi, 2011; Sennati et al., 2012). The Study for Monitoring Antimicrobial Resistance Trends (SMART) has been examining the activity of antimicrobials recommended for the treatment of IAIs globally since 2002. This report summarizes the susceptibility patterns and the etiology/characterization of beta-lactamases in *K. pneumoniae* isolated from patients with IAIs in Latin America in 2008–2012 as part of the SMART program.

2. Materials and methods

Between 2008 and 2012, 9744 gram-negative bacilli from IAIs, of which 1511 (15.5%) were *K. pneumoniae*, were isolated from 33 sites in 11 countries participating in the SMART program in Latin America: Argentina (2 sites), Brazil (6), Chile (2), Colombia (4), Dominican Republic (1), Ecuador (3), Guatemala (2), Mexico (4), Panama (3), Puerto Rico (3), and Venezuela (3). Each site collected up to 100 consecutive, nonselected isolates of aerobic or facultative anaerobic gram-negative bacilli from IAIs. Only 1 isolate per species per patient was allowed. All isolates were sent to a central laboratory (International Health Management Associates) for confirmation of identification, susceptibility testing, and molecular characterization of beta-lactamase (*bla*) genes. MICs and detection of ESBL activity were determined using custom dehydrated Microscan™ broth microdilution panels (Siemens Medical Solutions Diagnostics, West Sacramento, CA, USA) following manufacturer and Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2013). Isolates were classified as phenotypically ESBL positive if there was a ≥8-fold decrease in the MIC for ceftazidime or cefotaxime

* Corresponding author. Tel.: +1-847-303-5003; fax: +1-847-303-5601.
E-mail address: kkazmierczak@ihmainc.com (K.M. Kazmierczak).

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tested in combination with clavulanic acid compared to the MIC of ceftazidime or cefotaxime tested alone. MICs were interpreted using published CLSI guidelines (CLSI, 2013).

At least 50% of randomly chosen, phenotypically ESBL-positive *E. coli*, *K. pneumoniae*, *Klebsiella oxytoca*, and *Proteus mirabilis* and 97% of ertapenem-nonsusceptible (MIC >0.5 mg/L) Enterobacteriaceae isolates collected from each country during each year of the study were molecularly characterized. Genes encoding beta-lactamases of the TEM, SHV, CTX-M, CMY, DHA, FOX, MOX, ACC, MIR, ACT, KPC, OXA-48, NDM, VIM, and IMP types were detected using a combination of the Check-MDR CT101 microarray (Check-Points B.V., Wageningen, the Netherlands), as described previously (Hoban et al., 2012), and multiplex PCR assays, as described in Supplementary materials. Multiplex PCRs to detect bla_{IMP}, bla_{VIM}, and bla_{OXA-48} were performed for ertapenem-nonsusceptible isolates if bla_{KPC} or bla_{NDM} was not detected by microarray. Detection of bla_{VEB}, bla_{PER}, and bla_{GES} was performed on a subset of isolates in which other *bla* were not detected. Detected genes were sequenced and compared to public databases available from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) and the Lahey Clinic (www.lahey.org).

Before examining the molecularly characterized isolates for trends, the observed number of phenotypically ESBL-positive, ertapenem-susceptible (ESBL+, ERT-S) and ESBL-positive, ertapenem-nonsusceptible (ESBL+, ERT-NS) isolates with defined enzyme combinations were weighted using the respective yearly sampling fractions in order to estimate the true relative proportions of each enzyme combination. This correction was necessary because not all ESBL+, ERT-S or ESBL+, ERT-NS isolates collected in 2008–2012 were molecularly characterized, and the sampling fraction (i.e., the proportion of isolates that were characterized) varied from year to year. All statistical analyses were done using XLSTAT v2011.1.05. The Cochran–Armitage test was used to assess linear trends in percent susceptibility and percent phenotypically ESBL positive over time.

3. Results

Table 1 illustrates the percent susceptibility for all, phenotypically ESBL-positive and ESBL-negative *K. pneumoniae* for each year (2008–2012) versus amikacin, ampicillin-sulbactam, cefepime, cefotaxime, cefoxitin, ceftazidime, ceftriaxone, ciprofloxacin, ertapenem, imipenem, and piperacillin-tazobactam. ESBL-positive *K. pneumoniae* percentages varied by country (Supplementary Figure S1) and by year, ranging from 36.8% in 2009 to 48.1% in 2011 with an overall regional average of 42.4% over the 5 years studied. Trending analysis found no significant increase ($P > 0.05$) in phenotypically ESBL-positive *K. pneumoniae*. Statistical analysis measuring trends in susceptibility did demonstrate significant changes: the percent susceptibility for cefepime, ceftazidime, ceftriaxone, ertapenem, and imipenem versus all *K. pneumoniae* decreased significantly between 2008 and 2012 (with cefotaxime approaching significance, $P = 0.065$), and susceptibility for cefepime, cefotaxime, cefoxitin, ceftriaxone, ertapenem, and imipenem decreased among ESBL-positive isolates ($P < 0.05$). Although ampicillin-sulbactam showed significantly increasing susceptibility in ESBL-negative isolates ($P < 0.05$), it was the least active antimicrobial in vitro with percent susceptibility never exceeding 48% for *K. pneumoniae* overall. As expected, cefotaxime, ceftazidime, cefepime, and ceftriaxone all exhibited diminished activity against ESBL-positive isolates with percent susceptibility ≤14% in 2012. Due to the high proportion of ESBL-positive *K. pneumoniae* isolates in Latin America, the percent susceptibility of *K. pneumoniae* overall to these third- and fourth-generation cephalosporins did not exceed 63% in 2012. Ciprofloxacin, levofloxacin, and piperacillin-tazobactam also exhibited diminished activity against ESBL-positive *K. pneumoniae*, never exceeding 42% susceptible in this subset of isolates and never exceeding 73% susceptible for *K. pneumoniae* overall. Only cefoxitin, the aminoglycoside amikacin, and carbapenems ertapenem and imipenem retained susceptibility ≥66% against ESBL-positive isolates in 2012, as well as susceptibility ≥80% to *K. pneumoniae* overall.

Table 1
Percent susceptible for all, phenotypically ESBL-positive and ESBL-negative *K. pneumoniae* isolated in Latin American countries 2008–2012.

	% Susceptible *K. pneumoniae* (All | ESBL+ | ESBL−)				
Drug	2008	2009	2010	2011	2012
Amikacin	85 | 75 | 92	90 | 78 | 98	85 | 68 | 97	88 | 80 | 96	90 | 80 | 97
Ampicillin–Sulbactam	38 | 0 | 63	45 | 2 | 69	44 | 1 | 77	40 | 2 | 75	48 | 1 | 81**
Cefepime	64 | 15 | 97	71 | 24 | 99	62 | 12 | 99	52 | 5 | 95	62* | 12* | 98
Cefotaxime	59 | 5 | 95	65 | 9 | 97	58 | 5 | 98	49 | 1 | 94	58 | 3* | 97
Cefoxitin	79 | 66 | 87	85 | 82 | 87	82 | 71 | 89.6	74 | 59 | 88	80 | 66* | 90
Ceftazidime	65 | 19 | 97	69 | 19 | 98	62 | 14 | 98	53 | 10 | 94	63* | 14 | 97
Ceftriaxone	60 | 5 | 97	64 | 9 | 96	57 | 4 | 97	49 | 1 | 94	58* | 3* | 96
Ciprofloxacin	59 | 27 | 80	64 | 29 | 85	61 | 23 | 91	55 | 23 | 85	61 | 21 | 88
Ertapenem	94 | 89.8 | 97	96 | 91 | 98	90 | 79 | 99	84 | 73 | 94	86* | 68* | 98
Imipenem	97 | 95 | 98	98 | 97 | 99	93 | 86 | 99	89 | 81 | 95	87* | 73* | 97
Levofloxacin	66 | 41 | 83	68 | 38 | 85	67 | 34 | 92	62 | 36 | 86	68 | 37 | 90
Piperacillin–Tazobactam	68 | 37 | 89	73 | 42 | 91	66 | 28 | 94	63 | 31 | 92	72 | 40 | 94
n (All, ESBL+, ESBL−)	146 | 59 | 87	269 | 99 | 170	339 | 146 | 193	366 | 176 | 190	391 | 161 | 230
% ESBL+	40.4	36.8	43.1	48.1	41.2

% susceptible ≥90% shaded.
*Significant decreasing trend ($P < 0.05$).
**Significant increasing trend ($P < 0.05$).

A total of 516 *K. pneumoniae* collected in Latin America were molecularly characterized; of these, 352 were phenotypically ESBL positive and ertapenem susceptible, 141 were ESBL positive and ertapenem nonsusceptible, and 23 were ESBL negative and ertapenem nonsusceptible. A summary of genes encoding beta-lactamases (ESBL, AmpC, and carbapenemases only) and enzyme variants detected in each Latin American country is shown in Table 2 with observed enzyme group combinations shown in Table 3. Overall, isolates encoding only ESBLs comprised 71.7% of molecularly characterized isolates (Table 3). CTX-M-15, CTX-M-2, and SHV-12 were the ESBLs most frequently detected in Latin American countries and were widely disseminated. AmpC enzymes were detected in only 24 Latin American *K. pneumoniae* isolates. Of these, 15 isolates carried both AmpC and ESBL with the majority (11) collected in Panama (Tables 2, 3).

Genes encoding carbapenemases were detected in 113 of 164 (68.9%) ertapenem-nonsusceptible isolates and in 2 of 352 (0.6%) ertapenem-susceptible isolates (Table 3). KPC enzymes comprised 94.8% of the carbapenemases detected in ertapenem-nonsusceptible isolates, with only 5 OXA-48 family enzymes and 1 GES carbapenemase identified (Table 2). bla_{KPC} was detected in isolates collected from all countries except Chile, Dominican Republic, and Guatemala, whereas bla_{OXA-48} was detected in Argentina and Chile, and bla_{GES} was detected in Mexico. Of the ertapenem-nonsusceptible isolates, 25.0% carried KPC enzymes alone, whereas an additional 36.6% of isolates carried both KPC and an ESBL, most frequently SHV-12 or CTX-M-15. Six isolates (3.7%) collected in Panama coproduced KPC, AmpC, and ESBL beta-lactamases (Table 3 and Supplementary Table S1). No carbapenemase was identified in 51 of 164 (31.1%) ertapenem-nonsusceptible isolates. Forty-seven of these isolates encoded ESBLs (n = 45) or AmpC beta-lactamases (n = 2) and comprised the majority of ertapenem-nonsusceptible isolates characterized from Chile, Guatemala, and Mexico (Table 3 and Supplementary Table S2).

Trend analysis for Latin America overall showed that within the group of 641 phenotypically ESBL-positive isolates, the proportion of those producing only an ESBL or an ESBL with AmpC had decreased, whereas the proportion producing KPC or an ESBL and KPC had increased over the 5 years of this analysis (Table 4).

4. Discussion

Phenotypically ESBL-positive isolates comprised on average 42% of *K. pneumoniae* collected from IAIs as part of the SMART program in Latin America in 2008–2012. This proportion was much higher than observed for isolates collected from SMART investigators in other regions