International Journal of Infectious Diseases 35 (2015) 56–61

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

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

Susceptibility trends and molecular characterization of Gram-negative bacilli associated with urinary tract and intra-abdominal infections in Jordan and Lebanon: SMART 2011–2013

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ARTICLE INFO

Article history:
Received 11 February 2015
Received in revised form 27 March 2015
Accepted 18 April 2015

Keywords:
Resistance
Molecular characterization
Beta-lactamases
Intra-abdominal infection
Urinary tract infection
SMART

SUMMARY

Objectives: To investigate phenotypic and genotypic patterns of antimicrobial resistance among Gram-negative bacilli associated with urinary tract infection (UTI) and intra-abdominal infection (IAI) in medical centres of Jordan and Lebanon.
Methods: Gram-negative bacilli from the SMART study, collected between the years 2011 and 2013, were first identified at local laboratories. These isolates were shipped to a central laboratory where re-identification, susceptibility testing, and molecular characterization were performed using standard methods.
Results: Among the 523 UTI-associated isolates, *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* were the most frequent (70%, 14%, and 5%, respectively). *E. coli*, *K. pneumoniae*, and *Pseudomonas aeruginosa* were the most frequent species among the 527 IAI-associated isolates (46%, 14%, and 12%, respectively). Incidence rates of extended-spectrum beta-lactamase (ESBL) producers among UTI-associated *E. coli*, *K. pneumoniae*, and *P. mirabilis* were 43%, 54%, and 4%, respectively. Corresponding rates among IAI-associated isolates were 49%, 56%, and 12%, respectively. *Acinetobacter baumannii* and *P. aeruginosa* isolates showed very disturbing low susceptibility patterns. CTX-M-15 was the most prevalent ESBL produced. Seventeen isolates were non-susceptible to carbapenems (estimated prevalence of 1.6%).
Conclusions: The alarmingly high rates of ESBL production and emergence of carbapenemases emphasize the urgent need to develop antimicrobial stewardship initiatives and to maintain antimicrobial resistance surveillance systems.

1. Introduction

Healthcare-associated infections (HCAIs) continue to cause significant morbidity and mortality among hospitalized patients.[1] Gram-negative bacilli, especially in developing countries, are the most common and the most serious causes of these HCAIs.[2] The burden of these infections is complicated by trends of increasing antimicrobial resistance, complex clinical infections, and relatively fewer effective antimicrobials.[3] Patterns of antimicrobial resistance in Gram-negative bacilli are increasing alarmingly worldwide. These patterns include, among others, an increasing frequency of pathogens producing extended-spectrum beta-lactamases (ESBLs) and carbapenemases, including *Klebsiella pneumoniae* carbapenemases (KPCs). Among these patterns, ESBL producers continue to be the most common and they usually retain susceptibility to very few antimicrobials, such as carbapenems.[4]

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http://dx.doi.org/10.1016/j.ijid.2015.04.011

While large-scale surveillance systems are needed to identify dynamic patterns in ESBL producers worldwide, regional and local studies are equally important to provide evidence-based support to help develop local antimicrobial stewardship programmes.[5]

Jordan and Lebanon are Middle Eastern countries with many common characteristics. They share similar population demographics and healthcare system characteristics. Both have relatively open and liberal social systems, with widely available multilevel healthcare facilities and pharmaceutical options. It was therefore felt appropriate to combine data from the two countries in this report from the Study for Monitoring Antimicrobial Resistance Trends (SMART). The SMART programme generates data on the frequency, antimicrobial susceptibility, and ESBL rates of Gram-negative bacilli associated with urinary tract infections (UTI) and intra-abdominal infections (IAI) in participating medical centres in Jordan and Lebanon. It also highlights the molecular characterization for beta-lactam resistance.

2. Materials and methods

2.1. Setting and isolate collection

Hospital laboratories from the four participating sites (King Abdullah University Hospital and Jordan Hospital in Jordan, and Saint George University Hospital and Rodolphe Mérieux–Liban Laboratory in Lebanon) collected non-duplicate consecutive Gram-negative bacilli from patients with UTI and IAI from 2011 through 2013. The identification of isolates was performed according to the protocol of each participating laboratory. These isolates were then shipped to a central laboratory (International Health Management Associates, Inc., Schaumburg, IL, USA) for confirmation of identification and susceptibility testing.

2.2. Susceptibility testing

Antimicrobial susceptibility testing was done at the central laboratory using custom MicroScan dehydrated broth microdilution panels (Siemens Medical Solutions Diagnostics, West Sacramento, CA, USA). Minimum inhibitory concentrations (MICs) were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[6,7]

Antimicrobial panels included ertapenem (ETP), imipenem (IPM), cefepime (FEP), ceftazidime (CAZ), ceftazidime–clavulanic acid, cefoxitin (CFX), ciprofloxacin (CIP), amikacin (AMK), levofloxacin (LVX), cefotaxime (CTX), cefotaxime–clavulanic acid, piperacillin–tazobactam (TZP), ampicillin–sulbactam (SAM), and ceftriaxone (CRO), with concentrations as described previously.[8]

Isolates were classified as ESBL producers if there was at least an eight-fold reduction in the minimum inhibitory concentration for ceftazidime or cefotaxime tested in combination with clavulanic acid versus their MIC values when tested alone.

2.3. Quality control

Quality control testing (QC) was performed using the CLSI recommended American Type Culture Collection (ATCC) QC strains, as described previously.

2.4. Molecular characterization and strain typing

ESBLs and carbapenemases were characterized using the Check-Points microarray (Check-Points B.V., Wageningen, Netherlands), followed by PCR and sequencing. All *Enterobacteriaceae* that were non-susceptible to ertapenem (using CLSI breakpoints) were characterized; however, only 50% of the isolates that were phenotypically ESBL-positive but ertapenem-susceptible were characterized due to cost constraints. Therefore, 204 isolates were candidates for molecular characterization.

Three major groups of broad-spectrum beta-lactamases were distinguished and confirmed using recommended methods: extended-spectrum beta-lactamases (ESBLs), class C cephalosporinases (AmpC), and carbapenemases.

2.5. Statistical analysis

p-Values were calculated with confidence intervals set to 95%. *p*-Values of less than 0.05 were considered to indicate statistical significance. Data were analysed using PASW Statistics for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA).

2.6. Ethical considerations

Appropriate review board approvals were obtained as necessary. All data were kept confidential and patient identifying information was removed.

3. Results

A total of 1050 pathogens were isolated: 523 UTI-associated isolates and 527 from IAI. Among all species isolated, *Escherichia coli* and *Klebsiella pneumoniae* were the most frequently identified. A detailed description of the numbers and incidence rates of the species is given in Table 1.

The overall incidence rate of ESBLs among all *E. coli*, *K. pneumoniae*, and *Proteus mirabilis* isolates combined was 44%. The highest incidence of ESBL production occurred among IAI-associated *K. pneumoniae* isolates (56%), while the lowest was among UTI-associated *P. mirabilis* isolates (4%). No statistically significant differences were found between UTI and IAI ESBL incidences by species. Furthermore, these ESBL incidences did not increase significantly through the three consecutive study years.

Figures 1 and 2 compare susceptibility rates of ESBL-producing and non-producing isolates to 10 common antibiotics in both UTI- and IAI-associated infections. In general, ESBL-producing *E. coli* isolates of both infection groups had high susceptibility rates to imipenem, ertapenem, and amikacin, with no statistically significant differences compared to non-ESBL producers. However, corresponding rates for ESBL-producing *K. pneumoniae* were clearly lower. The drop in susceptibility rates among combined ESBL-producing *K. pneumoniae* isolates to imipenem (from 100% to 87.5%), ertapenem (from 100% to 87.5%), and amikacin (from 100% to 92.5%) was statistically significant ($p = 0.003$, 0.003, and 0.023, respectively).

Table 1
Gram-negative pathogens most frequently associated with UTI and IAI in Jordan and Lebanon SMART centres between 2011 and 2013

Pathogen	UTI			IAI		
	n	%	% ESBL	*n*	%	% ESBL
Escherichia coli	367	70	43	242	46	49
Klebsiella pneumoniae	71	14	54	75	14	56
Pseudomonas aeruginosa	17	3		65	12	
Proteus mirabilis	26	5	4	41	8	12
Enterobacter cloacae	7	1		29	6	
Acinetobacter baumannii	11	2		25	5	
Others	24	5		50	9	
Total	523	100		527	100	

UTI, urinary tract infections; IAI, intra-abdominal infections; SMART, Study for Monitoring Antimicrobial Resistance Trends; ESBL, extended-spectrum beta-lactamase.

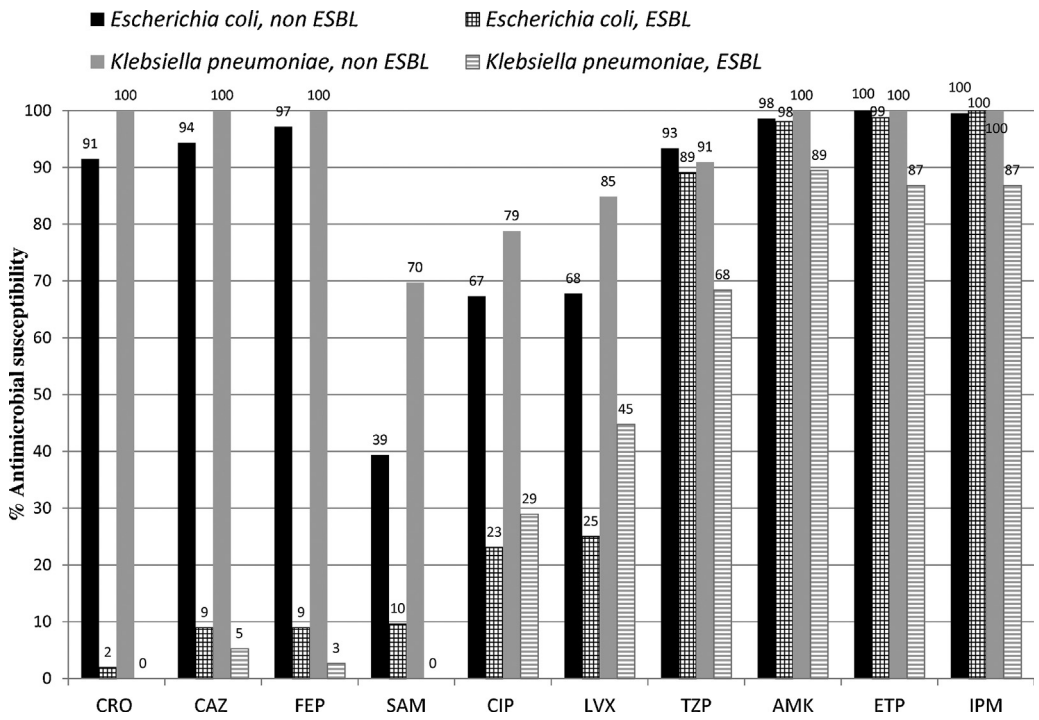

Figure 1. Antimicrobial susceptibility of *Escherichia coli* and *Klebsiella pneumoniae* (ESBL and non-ESBL producers) associated with UTI in Jordan and Lebanon SMART centres, 2011–2013. (CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; SAM, ampicillin–sulbactam; CIP, ciprofloxacin; LVX, levofloxacin; TZP, piperacillin–tazobactam; AMK, amikacin; ETP, ertapenem; IPM, imipenem).

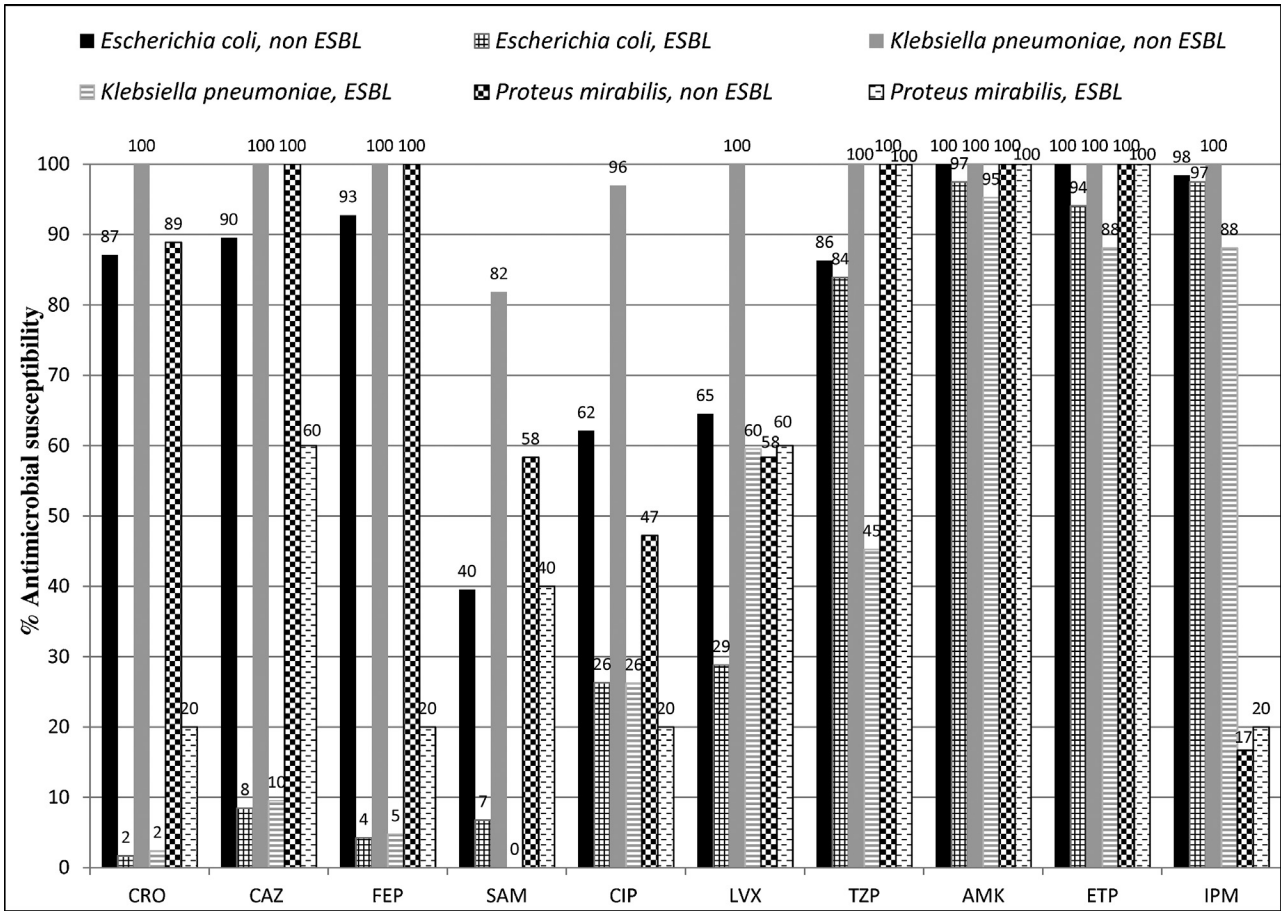

Figure 2. Antimicrobial susceptibility of *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* (ESBL and non-ESBL producers) associated with intra-abdominal infections in Jordan and Lebanon SMART centres, 2011–2013. (CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; SAM, ampicillin–sulbactam; CIP, ciprofloxacin; LVX, levofloxacin; TZP, piperacillin–tazobactam; AMK, amikacin; ETP, ertapenem; IPM, imipenem).

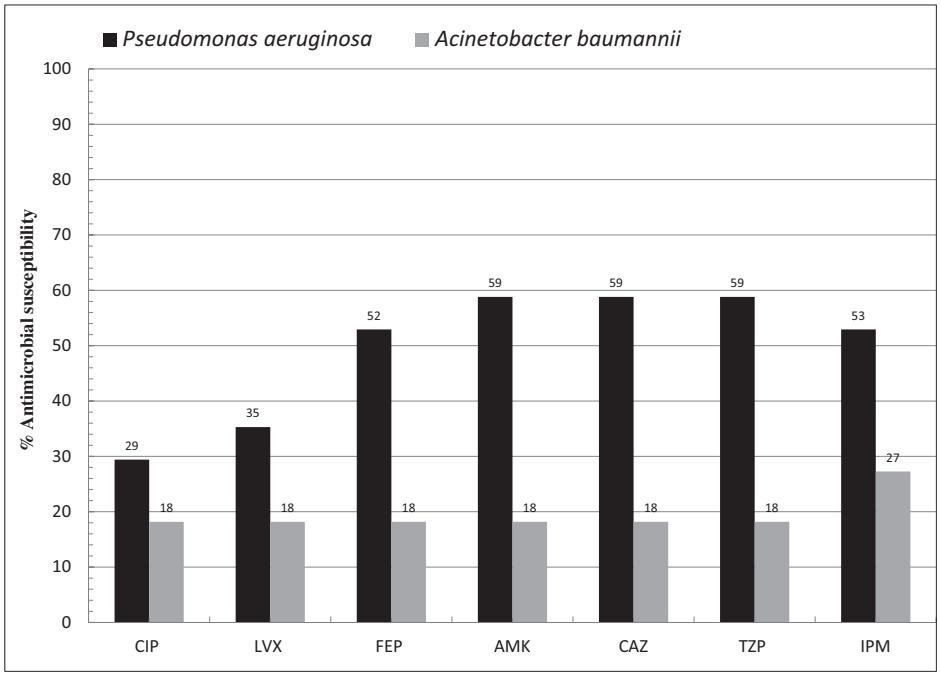

Figure 3. Antimicrobial susceptibility of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* associated with UTI in Jordan and Lebanon SMART centres, 2011–2013. (CIP, ciprofloxacin; LVX, levofloxacin; CAZ, ceftazidime; FEP, cefepime; AMK, amikacin; TZP, piperacillin–tazobactam; IPM, imipenem. The numbers reported on the histogram refer to the percentage of susceptibility).

In addition, ESBL producers showed low susceptibility rates to fluoroquinolones. Moreover, piperacillin–tazobactam had good activity against ESBL-producing *E. coli* (91% vs. 87%, $p = 0.127$), but had significantly diminished activity against ESBL-producing *K. pneumoniae* isolates (98% vs. 49%, $p < 0.0001$). All other tested antibiotics were mostly ineffective against both ESBL-producing *E. coli* and *K. pneumoniae* isolates.

Finally, both UTI- and IAI-associated *Acinetobacter baumannii* isolates showed very disturbing low susceptibility rates to various antibiotics (4% to 27%), with imipenem being the most active antibiotic. *Pseudomonas aeruginosa* showed better but still generally very low susceptibility rates (29% to 89%), with piperacillin–tazobactam being the most active antibiotic (Figures 3 and 4).

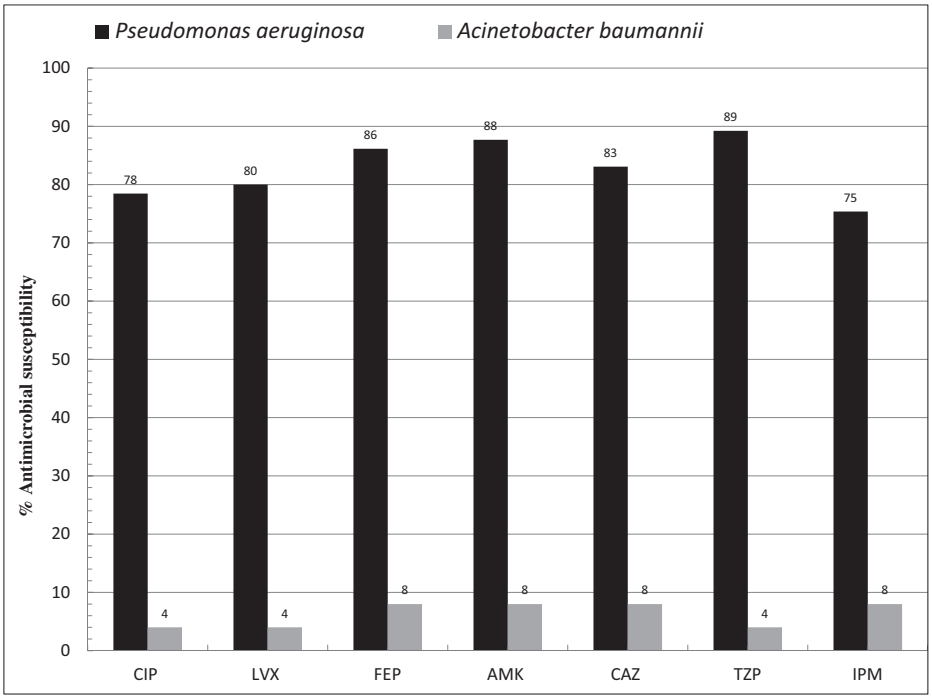

Figure 4. Antimicrobial susceptibility of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* associated with IAI in Jordan and Lebanon SMART centres, 2011–2013. (CIP, ciprofloxacin; LVX, levofloxacin; CAZ, ceftazidime; FEP, cefepime; AMK, amikacin; TZP, piperacillin–tazobactam; IPM, imipenem).

Molecular characterization was done for 204 ESBL-producing isolates and the ESBL distribution was as follows: (1) TEM-type beta-lactamases: TEM-169 (n = 1), TEM-33 (n = 1), TEM-52 (n = 1); (2) CTX-M-type beta-lactamases: CTX-M-15 (n = 178), CTX-M-14 (n = 5), CTX-M-27 (n = 4), CTX-M3 (n = 2), CTX-M-1 (n = 1), CTX-M-55 (n = 1), CTX-M-9 (n = 1), CTX-M-24 (n = 1); (3) SHV-type beta-lactamases: SHV-12 (n = 11), SHV-28 (n = 6), SHV-5 (n = 1); (4) other beta-lactamases: VEB-4 (n = 1). Many of these isolates produced multiple beta-lactamases (coexistence of two to four beta-lactamases), as shown in Table 2.

Fifteen isolates (n = 15) produced AmpC, with seven strains of *E. coli* and one of *K. pneumoniae* producing CMY-type AmpC and two strains of *Morganella morganii* and one *E. coli* producing DHA-type AmpC. Only one strain of *Enterobacter cloacae* and one of *Enterobacter asburiae* produced an ACT- and MIR-type AmpC, respectively. All these isolates produced other types of beta-lactamase.

Finally, 17 isolates (1.6%) were non-susceptible to carbapenems, with 10 producing OXA-48, seven producing NDM-1, and one KPC-2. These carbapenemase-producing isolates produced other beta-lactamases in conjunction.

Table 2
Genotypic distribution of various beta-lactamase combinations among 204 *Enterobacteriaceae* associated with UTI and IAI from Jordan and Lebanon SMART centres between 2011 and 2013

Organism	Molecular summary	Number of isolates
Escherichia coli (n = 145)	SHV-12; CTX-M-24; OXA-48	1
	CTX-M-15; OXA-48	2
	CTX-M-15; CMY	7
	CTX-M-1; DHA	1
	TEM-52; CTX-M-15	1
	TEM-33; CTX-M-1	1
	TEM-169; CTX-M-27	1
	SHV-12; CTX-M-14	1
	SHV-12; CTX-M-15	1
	SHV-12; KPC-2	1
	CTX-M-15	117
	CTX-M-14	4
	CTX-M-27	3
	CTX-M-3	1
	CTX-M-55	1
	SHV-12	2
Klebsiella pneumoniae (n = 49)	SHV-12; CTX-M-15; NDM-1; OXA-48	1
	SHV-12; CTX-M-15; CMY; OXA-48	1
	SHV-12; CTX-M-15; NDM-1	2
	SHV-28; CTX-M-15	6
	CTX-M-15; NDM-1	4
	CTX-M-15; OXA-48	2
	CTX-M-15	29
	SHV-5	1
	CTX-M-8	1
	CTX-M-9	1
	CTX-M-3	1
Enterobacter cloacae (n = 4)	ACT-type; OXA-48	1
	CTX-M-15; ACT-type	3
Klebsiella oxytoca (n = 2)	CTX-M-15	1
	OXA-48	1
Proteus mirabilis (n = 2)	CTX-M-15	1
	VEB-4	1
Morganella morganii (n = 1)	SHV-12; DHA; OXA-48	1
Enterobacter asburiae (n = 1)	MIR-type	1

UTI, urinary tract infections; IAI, intra-abdominal infections; SMART, Study for Monitoring Antimicrobial Resistance Trends.

4. Discussion

Previously published SMART data have shown that the Middle East has the second highest ESBL prevalence after Asia (around 37% for either UTI- or IAI-associated infections).[8] The current analysis showed that the combined UTI and IAI-associated ESBL prevalence was even higher in Jordan and Lebanon (42% and 46%, respectively). The Middle East is the only region in the world where the prevalence of ESBLs has been increasing significantly for both UTI- and IAI-associated infections,[8] which highlights the alarming patterns of these findings. These ESBL rates are even higher than those published in a study from a major tertiary care centre in Lebanon. That study showed a worrisome and remarkable increase in ESBL-producing *E. coli* isolates (4% in 2000 to 30% in 2011) and *K. pneumoniae* isolates (12% in 2000 to 28% in 2011).[9] A similar pattern has also been shown specifically for UTI-associated *E. coli* isolates from Lebanese patients, in whom the prevalence of ESBLs increased consistently from 2.3% in 2000 to 16.8% in 2009.[10] Another study from hospitals of north Jordan showed that the 2004 ESBL prevalence among *E. coli* and *K. pneumoniae* isolates was 10.8% and 71.4%, respectively.[11] An earlier study from the north of Jordan in 199 detected ESBLs in 34% of *K. pneumoniae* isolates.[12]

The high prevalence of ESBLs shown in the present report in both countries indicates that very limited antimicrobial options are available to treat infections caused by ESBL-producing *E. coli* and *K. pneumoniae* isolates, with carbapenems and amikacin remaining the only practical options among those studied in SMART to treat these infections. However, clinicians are usually reluctant to use amikacin alone to treat serious infections.

It is very important to emphasize that while carbapenems maintained excellent activity against ESBL-producing *E. coli* isolates during the study period, there was a significant drop in activity against ESBL-producing *K. pneumoniae* isolates (100% vs. 87.5%). This worrisome observation could be an indication of the presence of different emerging carbapenemase-encoding genes, as discussed further below.

While ESBL-producing isolates remained susceptible to imipenem, non-*Enterobacteriaceae* (*A. baumannii* and *P. aeruginosa*) revealed disappointing susceptibility rates to imipenem (14% and 71%, respectively). This trend presents a great challenge for any proposed antimicrobial stewardship programme. The rational use of carbapenems should be maximally practiced to treat individuals with ESBL-producing pathogens while trying to revive lost activity against *A. baumannii* and *P. aeruginosa*. This is where ertapenem (an antibiotic with *A. baumannii* and *P. aeruginosa* sparing activity) can be appropriately positioned.[13]

Molecular experiments revealed CTX-M-15 to be the most prevalent ESBL produced. Unlike most CTX-Ms that preferentially hydrolyze cefotaxime, CTX-M-15, an Asp-240-Gly variant of CTX-M-3, increases the catalytic efficiency against ceftazidime.[14] Our results are similar to those reported in a previous Lebanese study.[15]

SHV-12 was the most frequently detected ESBL in the remaining isolates. In contrast to the present study, among the SHV-type lactamases, SHV-5 and related enzymes appear to be the most prevalent ESBLs worldwide and have been responsible for outbreaks of nosocomial infection in several countries.[15–17]

Carbapenem-resistant Gram-negative bacilli have been reported worldwide as a consequence of the acquisition of carbapenemase genes. Outbreaks caused by carbapenemase genes have been reported in the Mediterranean region and they are even considered as endemic in some countries such as in Turkey and Italy where there is a high prevalence of OXA-48 and VIM-1, respectively.[18,19]

The overall prevalence of carbapenem-non-susceptible *Enterobacteriaceae* in Levant (the eastern part of the Mediterranean) from 2011 to 2013 was 1.6%. These results are in line with previously reported data in Lebanon that showed a prevalence of

1.2%.[20] If compared to the 4.2% reported by the US Centers for Disease Control and Prevention (CDC) in the year 2011, carbapenem resistance in *Enterobacteriaceae* in Levant appears modest.[21] Concerning the distribution of carbapenem resistance among enterobacterial species, the European network on carbapenemases reported in 2012 that carbapenemase producers in Europe are mainly identified among *K. pneumoniae* and *E. coli*.[22] The results of the present study are similar, as 14 out of 17 strains producing OXA-48 were *K. pneumoniae* and *E. coli*.

The carbapenemases reported in the present study were OXA-48, NDM-1, and KPC-2, the last two being isolated in Jordanian hospitals only. In Lebanon, different reports have described OXA-48, IMP-1, and NDM-1 in *Enterobacteriaceae* and OXA-58 in *A. baumannii*.[20,23–27] A very recent study[28] reported the carbapenemase genes bla_{OXA-23}, bla_{OXA-24}, bla_{GES-11}, bla_{VIM-2}, and bla_{IMP-2} for the first time in Lebanon. To the best of our knowledge, this is the first study reporting the KPC-2 gene in Levant.

In conclusion, this study constitutes the first report of SMART data for the Levant region. It showed ESBL rates to be alarmingly high and increasing among UTI- and IAI-associated *E. coli* and *K. pneumoniae* infections in both Jordan and Lebanon. While most ESBL-producing isolates were resistant to the majority of the antimicrobials evaluated in this study, they remained mostly susceptible to carbapenems (ertapenem and imipenem) and amikacin. However, ESBL-producing *K. pneumoniae* isolates were less susceptible to carbapenems than ESBL-producing *E. coli*. This trend is worrisome and can be explained by the molecular characterization results. Hence, these data show that even if the carbapenem-non-susceptible *Enterobacteriaceae* are moderately spread in Levant centres, the resistance depends predominantly on OXA-48 production especially identified in *K. pneumoniae* strains. In addition, antimicrobial options were almost absent for *A. baumannii* and were limited for *P. aeruginosa* isolates.

This study confirms the urgent need to continue surveillance programmes that monitor trends in antimicrobial activity and detect new resistance mechanisms as well as the spread of existing ones, and the need to develop antimicrobial stewardship initiatives, implement effective infection control programmes, and measure the effectiveness of such programmes in reducing or halting the spread of resistance, both regionally and globally.

Acknowledgements

We thank all of the SMART investigators in Jordan and Lebanon for their participation in this programme.

Funding: The SMART surveillance programme is funded by Merck & Co., Inc.

Conflict of interest: Robert Badal has served as a scientific consultant to Merck and received research support from Merck to conduct this study.

Contributions (transparency declarations): The authors are responsible for the work described in this paper. All authors were involved in at least one of the following: conception, design, acquisition, analysis, and statistical analysis, interpretation of data, and drafting the manuscript and/or revising the manuscript for important intellectual content. All authors provided final approval of the version to be published.

References

1. Michalopoulos A, Falagas ME, Karatza DC, Alexandropoulou P, Papadakis E, Gregorakos L, et al. Epidemiologic, clinical characteristics, and risk factors for adverse outcome in multiresistant Gram-negative primary bacteremia of critically ill patients. *Am J Infect Control* 2011;**39**:396–400.
2. Zowawi HM, Balkhy HH, Walsh TR, Paterson DL. Beta-lactamase production in key Gram-negative pathogen isolates from the Arabian Peninsula. *Clin Microbiol Rev* 2013;**26**:361–80.
3. Bassetti M, Merelli M, Temperoni C, Astilean A. New antibiotics for bad bugs: where are we? *Ann Clin Microbiol Antimicrob* 2013;**12**:22.
4. Hoban DJ, Lascols C, Nicolle LE, Badal R, Bouchillon S, Hackel M, et al. Antimicrobial susceptibility of *Enterobacteriaceae*, including molecular characterization of extended-spectrum beta-lactamase-producing species, in urinary tract isolates from hospitalized patients in North America and Europe: results from the SMART study 2009–2010. *Diagn Microbiol Infect Dis* 2012;**74**:62–7.
5. Hamilton KW, Fishman NO. Antimicrobial stewardship interventions: thinking inside and outside the box. *Infect Dis Clin North Am* 2014;**28**:301–13.
6. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standards. Document M07-A9. Ninth ed. Wayne, PA: CLSI; 2012.
7. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Document M100-S23. Wayne, PA: CLSI; 2013.
8. Morrissey I, Hackel M, Badal R, Bouchillon S, Hawser S, Biedenbach D. A review of ten years of the study for monitoring antimicrobial resistance trends (SMART) from 2002 to 2011. *Pharmaceuticals (Basel)* 2013;**6**:1335–46.
9. Araj GF, Avedissian AZ, Ayyash NS, Bey HA, El Asmar RG, Hammoud RZ, et al. A reflection on bacterial resistance to antimicrobial agents at a major tertiary care center in Lebanon over a decade. *J Med Liban* 2012;**60**:125–35.
10. Daoud Z, Afif C. *Escherichia coli* isolated from urinary tract infections of Lebanese patients between 2000 and 2009: epidemiology and profiles of resistance. *Chemother Res Pract* 2011;**2011**:218431.
11. Batchoun RG, Swedan SF, Shurman AM. Extended spectrum beta-lactamases among Gram-negative bacterial isolates from clinical specimens in three major hospitals in northern Jordan. *Int J Microbiol* 2009;**2009**:513874.
12. Youssef MT, Malkawi HI, Shurman AA, Andremont AO. Molecular typing of multiresistant *Klebsiella pneumoniae* isolated from children from northern Jordan. *J Trop Pediatr* 1999;**45**:271–7.
13. Yoon YK, Yang KS, Lee SE, Kim HJ, Sohn JW, Kim MJ. Effects of group 1 versus group 2 carbapenems on the susceptibility of *Acinetobacter baumannii* to carbapenems: a before and after intervention study of carbapenem-use stewardship. *PLoS One* 2014;**9**:e99101.
14. Poirel L, Gniadkowski M, Nordmann P. Biochemical analysis of the ceftazidime-hydrolysing extended-spectrum beta-lactamase CTX-M-15 and of its structurally related beta-lactamase CTX-M-3. *J Antimicrob Chemother* 2002;**50**:1031–4.
15. Moubareck C, Daoud Z, Hakime NI, Hamze M, Mangeney N, Matta H, et al. Countrywide spread of community- and hospital-acquired extended-spectrum beta-lactamase (CTX-M-15)-producing *Enterobacteriaceae* in Lebanon. *J Clin Microbiol* 2005;**43**:3309–13.
16. Mulgrave L, Attwood PV. Characterization of an SHV-5 related extended broad-spectrum beta-lactamase in *Enterobacteriaceae* from Western Australia. *Pathology* 1993;**25**:71–5.
17. Prodinger WM, Fille M, Bauernfeind A, Stemplinger I, Amann S, Pfausler B, et al. Molecular epidemiology of *Klebsiella pneumoniae* producing SHV-5 beta- lactamase: parallel outbreaks due to multiple plasmid transfer. *J Clin Microbiol* 1996;**34**:564–8.
18. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in *Klebsiella pneumoniae* and other *Enterobacteriaceae*: an evolving crisis of global dimensions. *Clin Microbiol Rev* 2012;**25**:682–707.
19. Glasner C, Albiger B, Buist G, Tambic Andrasevic A, Canton R, Carmeli Y, et al. Carbapenemase-producing *Enterobacteriaceae* in Europe: a survey among national experts from 39 countries, February 2013. *Euro Surveill* 2013;18. pii: 20525.
20. Hammoudi D, Moubareck CA, Aires J, Adaime A, Barakat A, Fayad N, et al. Countrywide spread of OXA-48 carbapenemase in Lebanon: surveillance and genetic characterization of carbapenem-non-susceptible *Enterobacteriaceae* in 10 hospitals over a one-year period. *Int J Infect Dis* 2014;**29C**:139–44.
21. Centers for Disease Control and Prevention (CDC). Vital signs: carbapenem-resistant *Enterobacteriaceae*. *MMWR Morb Mortal Wkly Rep* 2013;**62**:165–70.
22. Canton R, Akova M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, et al. Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. *Clin Microbiol Infect* 2012;**18**:413–31.
23. Baroud M, Dandache I, Araj GF, Wakim R, Kanj S, Kanafani Z, et al. Underlying mechanisms of carbapenem resistance in extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli* isolates at a tertiary care centre in Lebanon: role of OXA-48 and NDM-1 carbapenemases. *Int J Antimicrob Agents* 2013;**41**:75–9.
24. Daoud Z, Hobeika E, Choucair A, Rohban R. Isolation of the first metallo-beta-lactamase producing *Klebsiella pneumoniae* in Lebanon. *Rev Esp Quimioter* 2008;**21**:123–6.
25. El-Herte RI, Araj GF, Matar GM, Baroud M, Kanafani ZA, Kanj SS. Detection of carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* producing NDM-1 in Lebanon. *J Infect Dev Ctries* 2012;**6**:457–61.
26. Matar GM, Dandache I, Carrer A, Khairallah MT, Nordmann P, Sabra A, et al. Spread of OXA-48-mediated resistance to carbapenems in Lebanese *Klebsiella pneumoniae* and *Escherichia coli* that produce extended spectrum beta-lactamase. *Ann Trop Med Parasitol* 2010;**104**:271–4.
27. Zarrilli R, Vitale D, Di Popolo A, Bagattini M, Daoud Z, Khan AU, et al. A plasmid-borne blaOXA-58 gene confers imipenem resistance to *Acinetobacter baumannii* isolates from a Lebanese hospital. *Antimicrob Agents Chemother* 2008;**52**:4115–4120.
28. Hammoudi D, Ayoub Moubareck C, Kanso A, Nordmann P, Karam Sarkis D. Surveillance of carbapenem non-susceptible Gram-negative strains and characterization of carbapenemases of classes A, B, and D in a Lebanese hospital. J Med Liban 2015; in press.