Molecular characterization of beta-lactam resistant pathogens isolated from urinary infections in Lebanon and Jordan: An evaluation of SMART data 2010-2014.

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INTRODUCTION

The Study for Monitoring Antimicrobial Resistance Trends (SMART) has been following trends in resistance among gram-negative bacilli (GNB) since 2002. GNB resistance has been on the rise as shown by reports since then with a consistently increasing number of carbapenemases.

OBJECTIVE

This report summarizes the detection and molecular characterization of broad spectrum beta-lactamases in clinical isolates from GNB causing urinary tract infections (UTI) in Lebanon and Jordan. Five centers from Lebanon and Jordan participated during a 5 year period (from January 2010 to December 2014).

MATERIALS AND METHODS

733 isolates from UTI were collected from five participating centers in Lebanon (n=2) and Jordan (n=3). The prevalence of the isolated GNB from 2010 to 2014 as well as the prevalence of broad-spectrum beta-lactamases was assessed. Extended spectrum beta-lactamases (ESBLs) and carbapenemases were characterized using the Check-Points microarray (Check-Points B.V., Wageningen, The Netherlands), followed by PCR and sequencing. We characterized all *Enterobacteriaceae* that were non-susceptible to ertapenem (using CLSI breakpoints), and 50% (due to cost constraints) of the isolates that were phenotypically ESBL+ but ertapenem susceptible. Therefore, 159 isolates were candidates for molecular characterization. Three major groups of broad-spectrum beta-lactamases were distinguished and confirmed by recommended methods: ESBLs, class C cephalosporinases (AmpC), and carbapenemases.

RESULTS

Table 1 : Genotypic distribution of various beta-lactamase combinations among 159 *Enterobacteriaceae* associated with UTI from Lebanon and Jordan SMART centers between 2010 and 2014.

Organism	Molecular Summary	Number of isolates
Escherichia coli (n=126)	CTX-M-15	106
	CTX-M-14	4
	CTX-M-27	5
	CTX-M-8	1
	CTX-M-3	2
	SHV-12	2
	CTX-M-15; CTX-M-27	1
	CTX-M-15; CMY	1
	CTX-M-15; OXA-244	**1**
	SHV-12; CTX-M-15	1
	TEM-169; CTX-M-27	1
	CTX-M-15; OXA-48	**1**
Klebsiella pneumoniae (n=33)	CTX-M-15	20
	CTX-M-9	1
	CTX-M-3	1
	CTX-M-15; NDM-1	**4**
	SHV-2; CTX-M-15	1
	SHV-28; CTX-M-15	2
	CTX-M-15; OXA-181	**1**
	CTX-M-15; OXA-48	**1**
	CTX-M-14; OXA-48	**1**
	SHV-12; CTX-M-15; CMY; OXA-48	**1**

One hundred fifty-nine isolates (n=159) UTI isolates producing broad-spectrum beta-lactamases were detected. The distribution of the isolates was as follows:

- 126 *Escherichia coli*
- 33 *Klebsiella pneumoniae*

- Three major groups of broad-spectrum beta-lactamases were distinguished:
  - ❖ Extended-spectrum beta-lactamases (ESBLs)
  - ❖ Class C cephalosporinases (AmpC)
  - ❖ Carbapenemases.
- The distribution of the isolates as well as their molecular characteristics are shown in table 1.
- ESBL distribution was as presented in figure 1 (many isolates produced multiple beta-lactamases, with coexistence of two to four beta-lactamases).
- Only two isolates produced **CMY** type AmpC: one *E. coli* isolated in Lebanon and one *K. pneumoniae* isolated in Jordan. These isolates produced other types of beta-lactamases.
- Ten isolates (6.3%) were non-susceptible to carbapenems with four producing **OXA-48**, one **OXA-181**, one **OXA-244** and four **NDM-1** (marked in bold in Table 1). These carbapenemase-producing isolates also co-produced other beta-lactamases.

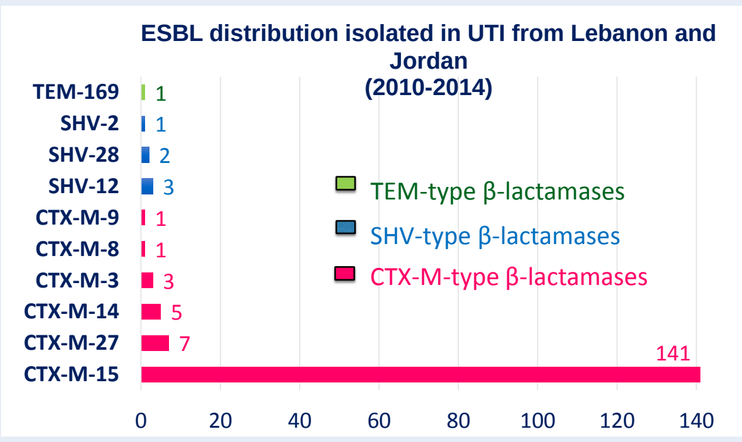

Figure 1 : ESBL distribution among 159 *Enterobacteriaceae* associated with UTI from Lebanon and Jordan SMART centers between 2010 and 2014.

DISCUSSION AND CONCLUSION

- Molecular experiments revealed that **CTX-M-15 was 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 [(1)]. Our results are similar to those reported in a previous Lebanese study [(2-4)]. In the remaining isolates, SHV-12 ESBL was the most frequently detected. Among the SHV-type-lactamases, unlike our study, SHV-5 and related enzymes seem to be the most prevalent ESBLs all over the world and were responsible for outbreaks of nosocomial infections in several countries [(2)].
- Carbapenem-resistant gram negative bacilli have been reported worldwide as a consequence of acquisition of carbapenemase genes. The reported carbapenemases in our study are **OXA-48**, **OXA-181**, **OXA-244** and **NDM-1.** In Lebanon, different reports described OXA-48, IMP-1 and NDM-1 in *Enterobacteriaceae* and OXA-58 in *A. baumannii* [(4-10)]. The overall **prevalence of carbapenem-non-susceptible *Enterobacteriaceae* in our study was 1.4 %.** These results are in line with previously reported data in Lebanon and Jordan that showed a prevalence of 1.6 % [(3)].

This study documented the molecular characterization of broad-spectrum beta-lactamases in clinical isolates from GNB in Lebanon and Jordan. Such studies are crucial in the continuous monitoring of local epidemiology to control the growth of this devastating problem of resistance.

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