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# 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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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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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.