

Infections caused by Gram negative bacteria in the unfortunate Syrian refugees in Jordan, epidemiological and molecular study

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Introduction

In Jordan, multidrug resistant organisms (MDROs=are resistant for three and more antibiotics from different antibiotic families) has been distributed widely in the last decade (Aqel et al., 2014). Carbapenems are used in that case to treat infections caused by MDROs (ChineseXDR et al., 2016) or ESBLs producer microbes. Carbapenemase producing *Enterobacteriaceae* (CPE) currently constitutes an important health problem causing variety of infections and treatment failure (Nordmann et al., 2011). Carbapenemase production (Class A: KPC, NMC, IMI, SME, and GES; Class B: IMP,VIM, NDM, GIM, SPM, and SIM; and Class D: OXA-48-like) is the main mechanism of resistance and have been described in *Enterobacteriaceae* (Manenzhe et al., 2015). CPE are distributed widely in many parts of the world. In 2014–2015 CPE considered endemic in countries like Turkey (mostly OXA-48 producers), Greece (mostly VIM producers) (Miriagou et al., 2010), Italy, Romania, northeastern USA, Zhejiang Province of China, and parts of the Middle East. However, lack of sanitation resulted from political conflicts such as wars might disturb infection control practices, and encourage the spread of MDROs.

In Jordan and the middle east region the isolation rates of CPE is found to range between 2–10 % (Aqel et al., 2016; Zowawi et al., 2014; Wartiti et al., 2012; Wadi et al., 2011). Jordan receives patients from other Arabian countries seeking different medical services, few studies have been investigated the prevalence rate of CPE and the outcomes of CPE infections. In Jordan, in the past five years, infections in the ICUs and surgery wards shows a degree of resistance to carbapenems (Aqel et al., 2016). Therefore, we conducted this study to detect MDROs (including CPE and ESBLs) and to determine their frequency with their antibiotic resistant gene characterization.

Materials and Methods

Bacterial isolates

During the year 2014 and 2015, 110 gram negative bacterial were isolated from clinical specimens (blood culture, urine, fluid, wound, tissue and sputum) taken from Jordanian and Syrian patients which were hospitalized in Al-Karak governmental hospital at Al-Karak area, Al-Mafraq hospital and other hospitals in Jordan in which were produced health services for Syrians. Isolates were identified and tested for susceptibility to a panel of antimicrobials (Vitek II, bioMérieux), the results for the latter were interpreted according the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2014. Species identification was confirmed using MALDI-TOF technique and other conventional tests. Medical history and patient's data were also collected.

Screening for ESBL and carbapenemase producing isolates

ESBLs production was determined with the double disk synergy test using amoxicillin-clavulanic acid in combination with cefotaxime, ceftazidime and cefepime (Aqel et al., 2014).

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Isolates with decreased susceptibility to ceftazidime MIC ≥ 16 $\mu\text{g/ml}$, ceftazidime MIC ≥ 8 $\mu\text{g/ml}$, cefotaxime MIC ≥ 2 $\mu\text{g/ml}$, imipenem MIC >1 mg/l , or meropenem MIC >1 mg/ml were investigated for carbapenemase activity (Amjad et al., 2011). Also, isolates with decreased susceptibility to ertapenem (zones of 21 mm or less around a 10 μg ertapenem disk) were investigated as possible carbapenemase producers according to CLSI guidelines (2014). The Modified Hodge Test (MHT) (Amjad et al., 2011) was performed on suspected carbapenemase producers according to CLSI guidelines (2014), with *K. pneumoniae* ATCC BAA-1705 and *K. pneumoniae* ATCC BAA-1706 used as positive and negative control strains, respectively.

Multiplex-PCRs for β -lactamase and carbapenemase genes

Crude DNA lysates were prepared by suspending a 1 μl loop-full of freshly cultured cells in 200 μL of sterile distilled water. The suspension was incubated for five minutes at 95°C and 1 μL of supernatant from the centrifuged lysates used as template DNA for PCR. Carbapenemase gene families (*bla*_{NDM}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{KPC}, *bla*_{OXA-48-like}) were detected via multiplex PCR and were determined according to Giakkoupi *et al.* (2013).

Results and Discussion

The most common infections in our patients were due to *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa* and *E. cloacae* with 62, 11, 7, 5, 3 rate of infections respectively (Table 1).

Despite the high value of carbapenems as an empirical treatment against serious infections due to ESBL producers, recent work has shown that 5.6% of the gram-negative isolates in Jordan were carbapenem resistant (Wadi et al., 2011). Here, our finding of carbapenemase production in 2.7% of 2014-15 isolates is lower than published rates in Europe or the USA, but does agree with reported carbapenemase rates from other Arabian countries (Zowawi et al., 2014; Poirel et al., 2012). Carbapenemase producers Enterobacteriaceae are limited in Jordan (Aqel et al., 2016). In our study, PCR amplification of the carbapenemase genes revealed that three of the meropenem resistant isolates harboured a *bla*_{VIM} gene. VIM carbapenemases were detected in different bacterial types, 1 (0.9%) for each isolate from *K. pneumoniae*, salmonella and *E. coli*. Detection of CPE VIM gene in Jordanian and Syrian patients (3/ 2.7%) from the same area represents clearly the dissemination of such isolates in both communities, either Syrian in the refugees camps or inside Jordanian community where Jordanian and Syrian live together (Poirel et al., 2012). The present antibiotic susceptibility data from the isolates clearly indicate the dissemination of broad-spectrum cephalosporin resistant Enterobacteriaceae, in our collection, 85 (77.2%) of the isolates were ESBLs producers. Enterobacteriaceae ESBL producers are well documented in Jordanian hospitals (Aqel et al., 2014). In our collection about 77% of the isolates were MDROs.

The isolates were resistant to amoxicillin/clavulanic acid, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cefotaxime, cefepime, ciprofloxacin and gentamicin in addition to the carbapenems. Susceptibility to tetracycline, amikacin and trimethoprim/sulfamethoxazole varied between the isolates. Isolates were susceptible to tigecycline and colistin. Most of the isolates showed a resistant pattern to meropenem (56.3%) phenotypically, but the resistant genes were detected in 3 isolates from the carbapenemase genes set screened in this study (Table 1). The methods we used for phenotypically detecting carbapenemase producers were according to the published standards that were current at the time, however updated methods for the detection of metallo-enzyme types as well as for ESBL enzymes will improve case ascertainment and thus the determined rate of carbapenemase producers still further. Nevertheless, the identification of these isolates harboring VIM enzyme types highlights the urgent need for continued study and surveillance of carbapenemase producers in Jordan,



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not least to improve the poor ascertainment of carbapenemase producers that is associated with standard front-line diagnostic laboratory workflows which do not specifically seek the detection of ESBL and/or carbapenemase phenotypes or genes (Arnold, et al., 2011).

Conclusions

MDROs including carbapenemase producer's and ESBLs gram negative bacteria are circulating in Jordanian hospital. Henceforth, infections of that nature may lead to high morbidity and mortality rate in Jordan, which will increase the medical and financial efforts to counter that microbes. Tigecycline and colistin both remained as solid treatment options against the carbapenemase producers we detected.

Finally, improved surveillance of resistance and the genetic determinants of resistance must now be a priority for multidrug resistant *Enterobacteriaceae* in Jordan and across our region. Adhering to extensive infection control measures in community and hospital-acquired infections will help disrupt the further spread of carbapenemase producing isolates among hospitalized patients.

Table 1. Isolates identification and other characteristics.

Maldi-Tof ID	Numbers	Jo ¹	Syr ²	Meropenem R ³	Resistance genes (VIM)
<i>E. coli</i>	67 (60.9)*	56	11	34	1 Syr
<i>E. aerogenes</i>	1 (0.9)	0	1	ND	
<i>E. cloacae</i>	3 (2.7)	3	0	3	
<i>K. pneumoniae</i>	11 (10)	10	1	10	1 Jo
<i>P. aeruginosa</i>	4 (3.6)	3	1	4	
<i>P. mirabilis</i>	9 (8.1)	9	0	9	
<i>Raoultella ornithinolytica</i>	1 (0.9)	1	0	1	
<i>Salmonella group</i>	1 (0.9)	0	1	ND	1 syr
<i>Other groups</i>	13 (11.8)	13	0	1	
Total	110	95 86.3%	15 13.6%	62 56.3%	3 2.7%

1, Jordanian; 2, Syrian; 3, not done; *, Percentage

References

- Amjad A, Mirza IA, Abbasi SA, Farwa U, Malik N, Zia F. Modified Hodge test: A simple and effective test for detection of carbapenemase production. *Iran J Microbiol* 2011; 3:189-93.
- Aqel AA, Meunier D, Alzoubi HM, Masalha IM, Wood-ford N. Detection of CTX-M-type extended-spectrum beta-lactamases among Jordanian clinical isolates of Enterobacteriaceae. *Scand J Infect Dis* 2014;46:155-7.
- Amin A. Aqel, Panagiota Giakkoupi, Hamed Alzoubi, Ibrahim Masalha, Matthew J. Ellington, Alkiviadis Vatopoulos. Detection of OXA-48-like and NDM carbapenemases producing *Klebsiellapneumoniae* in Jordan: A pilot study. *J Infect Public Health*. 2016 Mar 15. pii: S1876-0341(16)00038-1. doi: 10.1016/j.jiph.2016.02.002. [Epub ahead of print].
- Arnold RS, Thom KA, Sharma S, Phillips M, Kristie Johnson J, Morgan DJ. Emergence of *Klebsiella pneumoniae*



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This research was supported with a grant from the International Society for Infectious Diseases (ISID).

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carbapenemase-producing bacteria. *South Med J* 2011; 104:40-5.

5. Chinese XDR Consensus Working Group, X. Guan, L. He, B. Hu, J. Hu, X. Huang, G. Lai, et al. Laboratory diagnosis, clinical management and infection control of the infections caused by extensively drug-resistant Gram-negative bacilli: a Chinese consensus statement. *Clinical Microbiology and Infection*, Volume 22 Number S1, March 2016.
6. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; Nineteenth informational supplement. CLSI document, M100-S24. Wayne, PA, USA 2014.
7. Giakkoupi P, Tryfinopoulou K, Kontopidou F, Tsonou P, Golegou T, Souki H, et al. Emergence of NDM-producing *Klebsiella pneumoniae* in Greece. *Diagn Microbiol Infect Dis* 2013; 77:382-4.
8. Miriagou V, Pappagianistis CC, Tzelepi E et al. Detection of VIM-1 production in *Proteus mirabilis* by an imipenem dipicolinic acid double disk synergy test. *J Clin Microbiol* 2010; 4: 667-668.
9. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing *Enterobacteriaceae*. *Emerg Infect Dis* 2011; 17:1791-8
10. Poirel L, Bonnin RA, Nordmann P. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. *Antimicrob Agents Chemother* 2012; 56:559-62.
11. Rendani I, Manenzhe, Heather J, Zar, Mark P, Nicol I and Mamadou Kaba. The spread of carbapenemase-producing bacteria in Africa: a systematic review. *Antimicrob Chemother* 2015; 70: 23-40.
12. Wadi J, Haloub N, Al Ahmad M, Samara A, Romman A. Prevalence of meropenem susceptibility among Gram-negative pathogens isolated from intensive care units in Jordan. *Int Arab J Antimicrob Agents* 2011; 1(1:3).
13. Wartiti MA, Bahmani FZ, Elouennass M, Benouda A. Prevalence of carbapenemase-producing enterobacteriaceae in a University Hospital in Rabat, Morocco: a 19-months prospective study. *Int Arab J Antimicrob Agents* 2012; 2(3:4).
14. Zowawi HM, Sartor AL, Balkhy HH, Walsh TR, Al Johani SM, Aljindan RY, et al. Molecular characterization of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in the countries of the Gulf cooperation council: dominance of OXA-48 and NDM producers. *Antimicrob Agents Chemother* 2014; 58:3085-90.