HHS Public Access

Author manuscript

US Army Med Dep J. Author manuscript; available in PMC 2018 July 01.

Published in final edited form as:

US Army Med Dep J. 2017 ; (2-17): 12–17.

Low Prevalence of Carbapenem-Resistant Enterobacteriaceae among Wounded Military Personnel

Katrin Mende, PhD[a,b,c], **Miriam L. Beckius, MPH**[c], **Wendy C. Zera, BS**[a,b,c], **Fatma Onmus-Leone, MS**[d], **COL Clinton K. Murray, MC, USA**[c], and **David R. Tribble, MD, DrPH**[a] **on behalf of the Infectious Disease Clinical Research Program Trauma Infectious Disease Outcomes Study Group**

[a]Infectious Disease Clinical Research Program, Department of Preventive Medicine and Biostatistics, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

[b]The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, MD USA

[c]San Antonio Military Medical Center, Joint Base San Antonio, Fort Sam Houston, TX, USA

[d]Multidrug-resistant Organism Repository and Surveillance Network, Walter Reed Army Institute of Research, Silver Spring, MD, USA

Abstract

Multidrug-resistant organisms (MDROs) are a global health problem that impact both civilian and military populations. Among wounded warriors, MDROs further complicate the care of trauma-related infections, resulting in extended duration of hospitalization, as well as increased morbidity and mortality. During the wars in Iraq and Afghanistan, extended spectrum β-lactamase-producing Enterobacteriaceae were frequently isolated from wounded warriors. The potential emergence of difficult-to-treat carbapenem-resistant Enterobacteriaceae represented a serious challenge for clinicians. We examined carbapenem-resistant Enterobacteriaceae prevalence among wounded military personnel over a six-year period (2009–2015). Among 4090 Enterobacteriaceae isolates collected, 16 (0.4%) were carbapenem-resistant, of which the majority was *Enterobacter aerogenes* (44%) followed by *Klebsiella pneumoniae* (37%), and *Escherichia coli* (19%). Five isolates (31%) collected from two patients were carbapenemase-producers with one associated with an infection. All five carbapenemase-producing isolates were resistant to all tested carbapenems and each

Corresponding Author: Katrin Mende, PhD, San Antonio Military Medical Center, 3551 Roger Brooke Dr, MCHE-MDI, JBSA Fort Sam Houston, TX 78234. Phone: 210-916-3859; Fax: 210-916-0618; katrin.mende.ctr@mail.mil.

Conflict of Interest: The authors have no conflict of interest to declare.

Ethical standards: The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Disclaimer: The views expressed are those of the authors and do not necessarily reflect the official views of the Uniformed Services University of the Health Sciences, Henry M. Jackson Foundation for the Advancement of Military Medicine, the National Institutes of Health or the Department of Health and Human Services, Walter Reed Army Institute of Research, Brooke Army Medical Center, the U.S. Army Medical Department, the U.S. Army Office of the Surgeon General, the Department of Defense or the Departments of the Army, Navy or Air Force. Mention of trade names, commercial products, or organization does not imply endorsement by the US Government.

Role of the Funding Source: The sponsor of this study had no role in the study design, data analysis and/or interpretation, or writing the manuscript. The corresponding author had full access to the data and had final responsibility for the decision to submit for publication.

carried one carbapenemase gene (4 with blaKPC-3 and 1 with blaNDM-1). Overall, although a large number of Enterobacteriaceae isolates were collected, only a small proportion was carbapenem-resistant and data indicate a lack of a cluster. Due to these limited numbers, it is difficult to make any conclusions regarding the association between carbapenem resistance, antibiotic exposure, and clinical outcomes.

BACKGROUND

Colonization and infection with multidrug-resistant organisms, including extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, are a global health problem and frequently complicate the care of wounded military personnel.[1–6] Examination of surveillance cultures collected from wounded military personnel on admission to Landstuhl Regional Medical Center (LRMC) and military hospitals in the United States recovered 2065 colonizing isolates. The predominant organisms were *Escherichia coli* and *Klebsiella pneumoniae* (50% and 9%, respectively), of which 37% and 22%, were ESBL-producers.[7]

Recently, the emergence of carbapenem-resistant Enterobacteriaceae (CRE) has become an additional threat associated with difficult-to-treat infections, high mortality rates, and potential for wide transmission.[8–17] In an analysis of healthcare-associated infections in the United States, 2%, 2%, and 8% of *E. coli*, *Enterobacter* spp., and *K. pneumoniae* associated with surgical site infections were resistant to carbapenems, respectively.[8] The rising prevalence of CREs has further complicated patient care in the military health system. Surveillance of Department of Defense (DoD)-managed medical facilities within the United States and overseas reported a mean annual CRE incident rate of 0.49 per 100,000 patient-years. It was noted that the proportion of CRE increased from 0.033% in 2005 to 0.052% in 2012, with a steady rise in the proportion of carbapenem-resistant *Klebsiella* spp. between 2005 and 2010.[9] Furthermore, approximately 1% of *E. coli* and 8% of *K. pneumoniae* isolates recovered from U.S. military personnel and Afghan nationals treated at an U.S. deployed military hospital in Afghanistan were carbapenem-resistant.[18]

As more combat casualties are surviving grievous injuries, the rate of trauma-related infections has increased.[19] With complicated, polymicrobial wounds, the prevalence of multidrug-resistant organisms provides a challenge to clinicians treating wounded warriors. As carbapenems are often used to treat Gram-negative infections resistant to broad-spectrum antibiotics, the emergence of CREs is a cause for concern. As a result, we determined the prevalence of CREs among wounded military personnel and evaluated carbapenem resistance mechanisms.

METHODS

As part of the U.S. DoD – Department of Veterans Affairs, Trauma Infectious Disease Outcomes Study (TIDOS),[19] isolates were collected from military personnel injured during deployment and medically evacuated to LRMC (Germany) before being transferred to a participating military hospital in the United States (Walter Reed National Military Medical Center [National Naval Medical Center and Walter Reed Army Medical Center prior to September 2011] or San Antonio Military Medical Center [Brooke Army Medical Center

prior to September 2011]. All collected isolates were stored in a microbiological repository. Our analysis was restricted to Enterobacteriaceae isolates collected between June 1, 2009 and April 31, 2015. Isolates were classified as infecting if they were recovered from infection work-ups. Surveillance specimens were obtained from groin/axilla swabs performed within two days of hospital admission either at LRMC or the participating military hospitals in the United States. Multidrug resistance was defined as resistance to at least three of four antibiotic classes, ESBL production, or carbapenemase production.[20] For the purpose of our study, carbapenem resistance was defined as resistance to all carbapenems tested (i.e., meropenem, imipenem, doripenem, and ertapenem). Information related to infections, treatment, and outcomes was retrieved from the TIDOS infectious disease module,[19] which supplements the Department of Defense Trauma Registry.[21]

Isolate identities and antimicrobial susceptibilities were determined utilizing the BD Phoenix automated microbiology system and NMIC/ID-304 panels (BD Biosciences, Sparks, MD). As the BD Phoenix system does not report susceptibility results for certain bacteria/antibiotic combinations, E-test was also performed for all carbapenems.[22] Pulsed-field gel electrophoresis (PFGE) was conducted for genotyping in accordance with standard practices. Three multiplex PCRs for the detection of carbapenemase genes were performed using previously described primers (Table 1).[23] The PCRs were carried out with the following conditions: 5 minutes at 94ºC, 30 cycles of 30 seconds at 94ºC, 40 seconds at 56ºC, and 50 seconds at 72ºC, followed by 5 minutes at 72ºC.

The Neo-Rapid CARB Kit (Rosco Diagnostica, Taastrup, Denmark) was used to identify carbapenemase-producing isolates, which were sent to the Multidrug-Resistant Organism Repository and Surveillance Network (MRSN) for whole genome sequencing using the Illumina MiSeq platform. Paired-end sequencing of short (550 bp) and mate-paired sequencing of long (2–10 kb) genomic fragments was performed to obtain finished bacterial genomes. The genes encoding carbapenem resistance were identified along with other antibiotic resistance genes by BLASTN analysis using comprehensive web-based microbial annotation resources and pipelines developed internally by MRSN.

RESULTS

A total of 4090 Enterobacteriaceae isolates were collected from June 2009 through April 2015 (Table 2). Examination of antimicrobial susceptibility determined that 1391 isolates (34%) were multidrug-resistant and 1302 (32%) were classified as infecting. *E. coli* was the most common (51%), followed by *K. pneumoniae* (13%) and *Enterobacter cloacae* (12%).

A total of 141 isolates (3.4% of 4090) were resistant to at least one carabapenem; however, only 16 isolates (0.4% of 4090) were resistant to all tested carbapenems (100% resistant to doripenem, ertapenem, imipenem, and meropenem) with 50% associated with infections (Table 2). *Enterobacter aerogenes* (44%) was predominant, followed by *K. pneumoniae* (37%) and *E. coli* (19%). All carbapenem-resistant isolates were ESBL-producers. Twelve isolates (75%) were susceptible to amikacin, 9 (56%) to gentamycin, 5 (31%) to nitrofurantoin, 4 (25%) to tetracycline, and 1 (6%) to tobramycin. All *E. aerogenes* isolates were also susceptible to levofloxacin. The 16 isolates were recovered from 7 deployed

military personnel, of which 6 were wounded in Afghanistan and 1 was injured in Naples, Italy. Except for one isolate obtained from LRMC (Germany), the carbapenem-resistant isolates were collected from surveillance swabs or clinical cultures obtained at military hospitals in the United States (94%). The proportion of the 16 isolates varied annually, with 3 (19%) collected in 2009 (June–December), 4 (25%) in 2010, zero in 2011, 5 (31%) in 2012, 3 (19%) in 2013, and 1 (6%) in 2014.

Examination of PFGE results showed that all patients carried different strains of the Enterobacteriaceae organisms, indicating a lack of a cluster. Furthermore, no patients carried two or more different strains within the same genus. When serial isolates collected from patients were assessed, there were no genotypic changes.

Five *K. pneumoniae* isolates were carbapenemase-producers, of which four were from surveillance cultures (three from groin and one rectum) and one was associated with a pneumonia (collected from bronchoalveolar lavage). In addition, four were serial isolates from one patient collected at two separate facilities, including the infecting isolate recovered at Walter Reed National Military Medical Center three days after the patient's first positive groin culture at LRMC. One patient sustained a gunshot wound in the Afghanistan combat theater and the other was injured in a fall while stationed in Naples, Italy. The five carbapenemase-producing *K. pneumoniae* isolates were sent to MRSN for further testing and carbapenemase genes were identified. The PCR results found that one isolate carried the blaNDM gene, while the remaining four isolates from a single patient carried a blaKPC gene.

Whole genome sequencing of the five carbapenemase-producing *K. pneumoniae* isolates revealed that the four serial isolates (from the same patient) were genetically identical and represented a single clone (collected in 2012). Specifically, the four isolates belonged to MLST ST-258 and carried the carbapenemase gene blaKPC-3 on an approximately 78 kb plasmid that shared >98% homology to the previously described plasmid pKpQIL-LS6.[24] The single isolate from another patient belonged to MLST ST-11 (collected in 2014) and carried the carbapenemase gene blaNDM-1 on an approximately 73.5 kb plasmid that shared >95% homology to plasmid pS-300cz (Genbank Accession # KJ958927).

Two surveillance isolates amongst the serial carbapenemase-producing *K. pneumoniae* isolates from one patient were collected prior to treatment with meropenem, suggesting that the strain already carried the resistance gene and that meropenem exposure did not induce carbapenem resistance. For this patient, a carbapenem-susceptible *K. pneumoniae*, *Staphylococcus aureus* and *Acinetobacter baumannii-calcoaceticus* complex isolates were associated with the same pneumonia along with the carbapenemase-producing *K. pneumoniae*. Treating this patient with ampicillin-sulbactam, meropenem, and vancomycin cleared the infection during inpatient hospitalization.

DISCUSSION

Carbapenem-resistant Enterobacteriaceae are becoming more widespread in healthcare facilities in the United States and have been associated with high rates of mortality.[8–11,25,26]

While the rate of carbapenem resistance is still low, the rising prevalence is concerning. As a result, we examined isolates for carbapenem resistance collected from military personnel wounded during deployment in support of operations in Iraq and Afghanistan. Although a large number of Enterobacteriaceae isolates were collected from combat casualties over a study period of approximately six years, only 16 (0.4%) were carbapenem-resistant. The distribution of isolates across the study years and molecular typing findings indicate a lack of a carbapenem-resistant Enterobacteriaceae cluster with this wounded military population.

Prior to 2000, recovery of CREs was rare in the United States; however, within the past decade, an increase in the incidence of CREs has been observed. Specifically, according to the National Healthcare Safety Network, the proportion of CREs in U.S. acute care hospitals rose from 1.2% in 2001 to 4.2% in 2011 with *Klebsiella* spp. having the highest increases (1.6% to 10.4%, respectively).[25] Localized hospital outbreaks have also been reported in multiple states, including New York, Colorado, Illinois, and West Virginia.[26–31] It is notable that our analysis reports a lower proportion of CREs (0.4%) in combat casualties compared to findings from civilian U.S. hospitals, and may be the result of environmental factors or the approach to infection control.

Nonetheless, the proportion of CREs in our analysis is higher than previous surveillance reports from DoD-managed medical facilities (overall proportion of 0.055% over a period of 2005 to 2012). With regards to specific organisms, the proportion of carbapenem-resistant *E. coli*, *K. pneumoniae*, and *Enterobacter* spp. (*E. aerogenes* and *E. cloacae*) in our study (0.14%, 1.11%, and 0.81%, respectively) is also higher compared to the prior surveillance reports (0.041%, 0.116%, and 0.163%, respectively).[9]

Following recognition of increased carbapenem resistance due to widespread transmission of the blaNDM-1 gene, a military health system surveillance program was implemented in 2010, resulting in the screening by MRSN of all carbapenem-resistant isolates for the gene. Approximately 13 hospitals, including 5 in combat zones, submitted isolates for screening. In 2011, the first reported identification of the blaNDM-1 gene was identified in *Providencia stuartii* isolates recovered from an Afghan national burn patient treated at a U.S./coalition combat support hospital in Bagram, Afghanistan.[32,33] A low number of carbapenem-resistant *K. pneumoniae* isolates (4.2%) have also been collected from hospitals in Iraq; however, none were found to carry the blaNDM-1 or blaKPC-3 genes.[34]

Five (0.1% of all Enterobacteriaceae isolates; 32% of CRE isolates) carbapenemase-producing *K. pneumoniae* isolates were identified in our study. The five carbapenemase-producing isolates were recovered from two patients and one isolate was associated with an infection (i.e., pneumonia). All five isolates were resistant to all tested carbapenems and each isolate carried one carbapenemase gene, indicating that carbapenem resistance was due to the presence of the resistance genes. Specifically, four isolates carried blaKPC-3 while blaNDM-1 was only identified with one isolate. The patient with the isolates carrying blaKPC-3 sustained injuries in Naples, Italy, and was treated initially at a Naples hospital. As a carbapenem-resistant ST258 *K. pneumoniae* strain carrying blaKPC-3 has been previously reported in Italy,[24] there is the potential that the carbapenemase-producing *K. pneumoniae* isolates were acquired through hospital transmission by the injured service

member. To the best of our knowledge, this is the first report of *K. pneumoniae* isolates carrying blaNDM-1 recovered from military personnel wounded in Afghanistan.

Due to the low numbers of CREs in our study, it is difficult to analyze the association between carbapenem resistance, antibiotic exposure, and clinical outcomes. It is also difficult to draw any conclusions regarding transmission patterns of CREs in this patient population. Nevertheless, as CREs are becoming widespread in both civilian and military health systems, surveillance should continue.

Acknowledgments

We are indebted to the Infectious Disease Clinical Research Program Trauma Infectious Disease Outcomes Study team of clinical coordinators, microbiology technicians, data managers, clinical site managers, and administrative support personnel for their tireless hours to ensure the success of this project.

Financial Support: Support for this work (IDCRP-024) was provided by the Infectious Disease Clinical Research Program, a Department of Defense program executed through the Uniformed Services University of the Health Sciences, Department of Preventive Medicine and Biostatistics. This project has been funded by the Global Emerging Infections Surveillance and Response System (GEIS), a Division of the Armed Forces Health Surveillance Center (AFHSC) and the National Institute of Allergy and Infectious Diseases, National Institutes of Health, under Inter-Agency Agreement Y1-AI-5072, and the Department of the Navy under the Wounded, Ill, and Injured Program.

References

1. Hospenthal DR, Crouch HK, English JF, Leach F, Pool J, Conger NG, Whitman TJ, Wortmann GW, Robertson JL, Murray CK. Multidrug-resistant bacterial colonization of combat-injured personnel at admission to medical centers after evacuation from Afghanistan and Iraq. J Trauma Acute Care Surg. 2011; 71:S52–S57.
2. Mende K, Beckius ML, Zera WC, Yu X, Cheatle KA, Aggarwal D, Li P, Lloyd BA, Tribble DR, Weintrob AC, Murray CK. Phenotypic and genotypic changes over time and across facilities of serial colonizing and infecting *Escherichia coli* isolates recovered from injured service members. J Clin Microbiol. 2014; 52:3869–3877. [PubMed: 25143566]
3. Murray CK, Roop SA, Hospenthal DR, Dooley DP, Wenner K, Hammock J, Taufen N, Gourdine E. Bacteriology of war wounds at the time of injury. Mil Med. 2006; 171:826–829. [PubMed: 17036599]
4. Hospenthal DR, Crouch HK, English JF, Leach F, Pool J, Conger NG, Whitman TJ, Wortmann GW, Murray CK, Cordts PR, Gamble WB. Response to infection control challenges in the deployed setting: Operations Iraqi and Enduring Freedom. J Trauma. 2010; 69(Suppl 1):S94–S101. [PubMed: 20622627]
5. Keen EF 3rd, Murray CK, Robinson BJ, Hospenthal DR, Co EM, Aldous WK. Changes in the incidences of multidrug-resistant and extensively drug-resistant organisms isolated in a military medical center. Infect Control Hosp Epidemiol. 2010; 31:728–732. [PubMed: 20500036]
6. Scott P, Deye G, Srinivasan A, Murray C, Moran K, Hulten E, Fishbain J, Craft D, Riddell S, Lindler L, Mancuso J, Milstrey E, Bautista CT, Patel J, Ewell A, Hamilton T, Gaddy C, Tenney M, Christopher G, Petersen K, Endy T, Petruccelli B. An outbreak of multidrug-resistant *Acinetobacter baumannii-calcoaceticus* complex infection in the US military health care system associated with military operations in Iraq. Clin Infect Dis. 2007; 44:1577–1584. [PubMed: 17516401]
7. Weintrob AC, Murray CK, Lloyd BA, Li P, Lu D, Miao Z, Aggarwal D, Carson ML, Gaskins LJ, Tribble DR. Active surveillance for asymptomatic colonization with multidrug-resistant gram negative bacilli among injured service members - a three year evaluation. MSMR. 2013; 20:17–22.
8. Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, Kallen A, Limbago B, Fridkin S. for the National Healthcare Safety Network (NHSN) Team and Participating NHSN Facilities. Antimicrobial-resistant pathogens associated with healthcare-associated infections: Summary of

data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. Infect Control Hosp Epidemiol. 2013; 34:1–14. [PubMed: 23221186]

9. Lesho EP, Clifford RJ, Chukwuma U, Kwak YI, Maneval M, Neumann C, Xie S, Nielsen LE, Julius MD, McGann P, Waterman PE. Carbapenem-resistant Enterobacteriaceae and the correlation between carbapenem and fluoroquinolone usage and resistance in the US military health system. Diagn Microbiol Infect Dis. 2015; 81:119–125. [PubMed: 25497458]
10. Falagas ME, Lourida P, Poulikakos P, Rafailidis PI, Tansarli GS. Antibiotic treatment of infections due to carbapenem-resistant Enterobacteriaceae: Systematic evaluation of the available evidence. Antimicrob Agents Chemother. 2014; 58:654–663. [PubMed: 24080646]
11. Falagas ME, Tansarli GS, Karageorgopoulos DE, Vardakas KZ. Deaths attributable to carbapenem-resistant Enterobacteriaceae infections. Emerg Infect Dis. 2014; 20:1170–1175. [PubMed: 24959688]
12. Doi Y, Paterson DL. Carbapenemase-producing Enterobacteriaceae. Semin Respir Crit Care Med. 2015; 36:74–84. [PubMed: 25643272]
13. Lazarovitch T, Amity K, Coyle JR, Ackerman B, Tal-Jasper R, Ofer-Friedman H, Hayakawa K, Bogan C, Lephart PR, Kaplansky T, Maskit M, Azouri T, Zaidenstein R, Perez F, Bonomo RA, Kaye KS, Marchaim D. The complex epidemiology of carbapenem-resistant Enterobacter infections: a multicenter descriptive analysis. Infect Control Hosp Epidemiol. 2015; 36:1283–1291. [PubMed: 26399907]
14. Kim SR, Rim CB, Kim Y, Kim JW, Song YW, Shin SH, Yoon HJ, Shim S. Four cases of carbapenem-resistant Enterobacteriaceae infection from January to March in 2014. Korean J Fam Med. 2015; 36:191–194. [PubMed: 26217484]
15. Guh AY, Bulens SN, Mu Y, Jacob JT, Reno J, Scott J, Wilson LE, Vaeth E, Lynfield R, Shaw KM, Vagnone PM, Bamberg WM, Janelle SJ, Dumyati G, Concannon C, Beldavs Z, Cunningham M, Cassidy PM, Phipps EC, Kenslow N, Travis T, Lonsway D, Rasheed JK, Limbago BM, Kallen AJ. Epidemiology of carbapenem-resistant Enterobacteriaceae in 7 US communities, 2012–2013. JAMA. 2015; 314:1479–1487. [PubMed: 26436831]
16. Mayer CL, Haley VB, Giardina R, Hazamy PA, Tsivitis M, Knab R, Lutterloh E. Lessons learned from initial reporting of carbapenem-resistant Enterobacteriaceae in New York State hospitals, 2013–2014. Am J Infect Control. 2016; 44:131–133. [PubMed: 26601706]
17. Deutsch RF, Ross JW, Nailor MD. Carbapenem-resistant Enterobacteriaceae: a case series of infections at Hartford Hospital. Conn Med. 2015; 79:269–275. [PubMed: 26245014]
18. Sutter DE, Bradshaw LU, Simkins LH, Summers AM, Atha M, Elwood RL, Robertson JL, Murray CK, Wortmann GW, Hospenthal DR. High incidence of multidrug-resistant gram-negative bacteria recovered from Afghan patients at a deployed US military hospital. Infect Control Hosp Epidemiol. 2011; 32:854–860. [PubMed: 21828965]
19. Tribble DR, Conger NG, Fraser S, Gleeson TD, Wilkins K, Antonille T, Weintrob A, Ganesan A, Gaskins LJ, Li P, Grandits G, Landrum ML, Hospenthal DR, Millar EV, Blackbourne LH, Dunne JR, Craft D, Mende K, Wortmann GW, Herlihy R, McDonald J, Murray CK. Infection-associated clinical outcomes in hospitalized medical evacuees after traumatic injury: Trauma Infectious Disease Outcome Study. J Trauma Acute Care Surg. 2011; 71:S33–S42.
20. Division of Healthcare Quality Promotion. Protocol multidrug-resistant organism and Clostridium difficile Infection (MDRO/CDI) module. Centers for Disease Control and Prevention; Atlanta, GA: 2016. The National Healthcare Safety Network (NHSN) Manual. Patient safety component.
21. Eastridge BJ, Jenkins D, Flaherty S, Schiller H, Holcomb JB. Trauma system development in a theater of war: Experiences from Operation Iraqi Freedom and Operation Enduring Freedom. J Trauma. 2006; 61:1366–1372. [PubMed: 17159678]
22. Clinical and Laboratory Standards Institute. Analysis and presentation of cumulative antimicrobial susceptibility test data; approved guideline – Third Edition. Wayne, PA: 2009. CLSI document M39-A3
23. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis. 2011; 70:119–123. [PubMed: 21398074]
24. Villa L, Capone A, Fortini D, Dolejska M, Rodriguez I, Taglietti F, De Paolis P, Petrosillo N, Carattoli A. Reversion to susceptibility of a carbapenem-resistant clinical isolate of *Klebsiella*

pneumoniae producing KPC-3. J Antimicrob Chemother. 2013; 68:2482–2486. [PubMed: 23800906]

25. Centers for Disease Control and Prevention. Vital signs: Carbapenem-resistant Enterobacteriaceae. MMWR Morb Mortal Wkly Rep. 2013; 62:165–170. [PubMed: 23466435]
26. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant Enterobacteriaceae epidemiology and prevention. Clin Infect Dis. 2011; 53:60–67. [PubMed: 21653305]
27. Frias M, Tsai V, Moulton-Meissner H, Avillan J, Hunter J, Arwady MA, Epstein L. Notes from the field: New Delhi metallo-β-lactamase-producing *Escherichia coli* associated with endoscopic retrograde cholangiopancreatography - Illinois, 2013. MMWR Morb Mortal Wkly Rep. 2014; 62:1051. [PubMed: 24381080]
28. Gaviria D, Greenfield V, Bixler D, Thomas CA, Ibrahim SM, Kallen A, Limbago B, Kitchel B, Taylor TK. Carbapenem-resistant *Klebsiella pneumoniae* associated with a long-term--care facilitiy ---West Virginia, 2009--2011. MMWR Morb Mortal Wkly Rep. 2011; 60:1418–1420. [PubMed: 22012114]
29. Pisney L, Barron M, Jackson S, Bamberg W, MacCannell D, Kallen A, Gould C, Limbago B, Epson E, Wendt J. Notes from the field: Hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* producing New Delhi metallo-beta-lactamase -Denver, Colorado, 2012. MMWR Morb Mortal Wkly Rep. 2013; 62:108. [PubMed: 23407128]
30. Epstein L, Hunter JC, Arwady MA, Tsai V, Stein L, Gribogiannis M, Frias M, Guh AY, Laufer AS, Black S, Pacilli M, Moulton-Meissner H, Rasheed JK, Avillan JJ, Kitchel B, Limbago BM, MacCannell D, Lonsway D, Noble-Wang J, Conway J, Conover C, Vernon M, Kallen A. New Delhi metallo-beta-lactamase-producing carbapenem-resistant *Escherichia coli* associated with exposre to duodenoscopes. JAMA. 2014; 312:1447–1455. [PubMed: 25291580]
31. Bratu S, Landman D, Haag R, Recco R, Eramo A, Alam M, Quale J. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: A new threat to our antibiotic armamentarium. Arch Intern Med. 2005; 165:1430–1435. [PubMed: 15983294]
32. Storey S, McGann PT, Lesho EP, Waterman PE. Notes from the field: Detection of bla_{NDM-1} carbapenem resistance in a clinical isolate of Providencia stuartii in a U.S./coalition medical facility ---Afghanistan, 2011. MMWR Morb Mortal Wkly Rep. 2011; 60:756.
33. McGann P, Hang J, Clifford RJ, Yang Y, Kwak YI, Kuschner RA, Lesho EP, Waterman PE. Complete sequence of a novel 178-kilobase plasmid carrying blaNDM-1 in a *Providencia stuartii* strain isolated in Afghanistan. Antimicrob Agents Chemother. 2012; 56:1673–1679. [PubMed: 22290972]
34. Al Sehlawi ZS, Almohana AM, AlThabab AA. Occurrence and detection of carbapenemase-producing *Klebsiella pneumoniae* clinical isolates in Najaf hospitals. Al-Kufa J Biol. 2013; 5:e321.

Table 1

PCR Oligonucleotide Primers for the Amplification of Carbapenemase Genes

Gene	Primer Sequence (5′-3′)	Product Size (bp)
blaIMP	F: GGAATAGAGTGGCTTAAYTCTC R: GGTTTAAYAAAACAACCACC	232
blaSPM	F: AAAATCTGGGTACGCAAACG R: ACATTATCCGCTGGAACAGG	271
blaAIM	F: CTGAAGGTGTACGGAAACAC R: GTTCGGCCACCTCGAATTG	322
blaVIM	F: GATGGTGTTTGGTCGCATA R: CGAATGCGCAGCACCAG	390
blaOXA	F: GCGTGGTTAAGGATGAACAC R: CATCAAGTTCAACCCAACCG	438
blaGIM	F: TCGACACACCTTGGTCTGAA R: AACTTCCAACTTTGCCATGC	477
blaBIC	F: TATGCAGCTCCTTTAAGGGC R: TCATTGGCGGTGCCGTACAC	537
blaSIM	F: TACAAGGGATTCGGCATCG R: TAATGGCCTGTTCCCATGTG	570
blaNDM	F: GGTTTGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC	621
blaDIM	F: GCTTGTCTTCGCTTGCTAACG R: CGTTCGGCTGGATTGATTTG	699
blaKPC	F: CGTCTAGTTCTGCTGTCTTG R: CTTGTCATCCTTGTTAGGCG	798

Table 2

Most Common Enterobacteriaceae Collected from Wounded Military Personnel (2009–2014) with a Focus on Carbapenem Resistance

Organisms[a]	Surveillance (%)	Infecting (%)	Total (%)
Escherichia coli	1608 (57.7)	495 (38.0)	2103 (51.4)
Resistant to ≥1 carbapenem	12 (0.7)	9 (1.8)	21 (1.0)
Carbapenem-resistant *E. coli*[b]	**0**	**3 (0.6)**	**3 (0.1)**
Klebsiella pneumoniae	391 (14.0)	150 (11.5)	541 (13.2)
Resistant to ≥1 carbapenem	15 (3.8)	7 (4.7)	22 (4.1)
Carbapenem-resistant *K. pneumoniae*[b]	**5 (1.3)**	**1 (0.7)**	**6 (1.1)**
Enterobacter cloacae	227 (8.1)	284 (21.8)	511 (12.5)
Resistant to ≥1 carbapenem	6 (2.6)	6 (2.1)	12 (2.3)
Carbapenem-resistant *E. cloacae*[b]	**0**	**0**	**0**
Enterobacter aerogenes	232 (8.3)	125 (9.6)	357 (8.7)
Resistant to ≥1 carbapenem	17 (7.3)	20 (16.0)	37 (10.4)
Carbapenem-resistant *E. aerogenes*[b]	**3 (1.3)**	**4 (3.2)**	**7 (2.0)**
Serratia marcescens	76 (2.7)	120 (9.2)	196 (4.8)
Resistant to ≥1 carbapenem	3 (3.9)	14 (11.7)	17 (8.7)
Carbapenem-resistant *S. marcescens*[b]	**0**	**0**	**0**
Total Enterobacteriaceae[c]	2788	1302	4090
Total Enterobacteriaceae resistant to ≥1 carbapenem	70 (2.5)	71 (5.5)	141 (3.4)
Total Carbapenem-Resistant Enterobacteriaceae[b]	**8 (0.3)**	**8 (0.6)**	**16 (0.4)**

[a]The percentage of carbapenem-resistant isolates for each organism is calculated using the totals for that specific organism

[b]Carbapenem-resistant Enterobacteriaceae are defined as being resistant to all tested carbapenems (i.e., meropenem, imipenem, doripenem, and ertapenem)

[c]Only the top five organisms are presented so the total for the overall Enterobacteriaceae is greater than the sum of the columns.