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High Prevalence of Multidrug-Resistant Bacteria in Libyan War Casualties Admitted to a Tertiary Care Hospital, Germany

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The ongoing Libyan conflict constantly causes victims among the military and civilian population. Cross-border transfer of patients represents a high risk of introducing multidrug-resistant organisms (MDROs), for example, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci, and carbapenem-resistant gram-negative organisms (CROs), into the country of destination. This study assessed the MDRO status in Libyan war casualties ($n=67$) admitted to Northwest Medical Centre in Frankfurt/Main, Germany, from August 2016 till January 2017. Identified multidrug-resistant nonfermenters and Enterobacteriaceae were subjected to molecular detection of β-lactamases and further mechanisms of resistance. All isolates were typed by enzymatic macrorestriction and subsequent pulsed-field gel electrophoresis. MDROs were found in 40 (60%) patients, including 25 (37%) positive for at least one CRO and 11 (16%) patients with MRSA. A total of 37 isolates of *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Escherichia coli*, *Enterobacter cloacae*, and *Serratia marcescens* produced carbapenemases: NDM ($n=17$), OXA-48 ($n=15$), and OXA-23 ($n=9$) in addition to other β-lactamases (with $bla_{\text{CTX-M-group-1}}$ being most frequent) and plasmid-mediated quinolone resistance genes (*qnrB, aac(6′)Ib-cr*). Bacterial strain typing revealed the presence of various clones. This high MDRO rate in Libyan war casualties demands awareness, appropriate screening, and containment measures for medical institutions involved in medical care to avoid patient-to-patient transmission.

Keywords: multidrug resistance, carbapenemase, NDM, *Acinetobacter baumannii*, Enterobacteriaceae, OXA

Introduction

While the world's attention is mostly focused on the ongoing Syrian civil war and refugee crisis, the continuing armed conflict in Libya seems forgotten. The UN and ICC prosecutors have estimated that ~20,000 civilians are injured annually in Libya alone, and that at least 600 were killed in 2015.[1] After receiving preliminary treatment in local healthcare facilities, many of the severely injured are transferred to European hospitals for further medical aid.[2,3] Similar to some parts of Europe[4] and many other regions of the world,[5] the rising problem of antibiotic resistance in gram-negative bacteria is highly evident in most North African hospitals and has a severe impact on local healthcare.[6–8] Likewise, methicillin-resistant *Staphylococcus aureus* (MRSA) colonization rates of Libyan healthcare workers (11–37%), patients (12–55%), and the general population (10%) are persistently high.[6,9,10]

Therefore, medical cross-border transfer of patients from local hospitals to European medical centers poses a complex risk to healthcare facilities in the destination country due to the continuous introduction of multidrug-resistant organisms (MDROs), including carbapenem-resistant gram-negative organisms (CROs).

Herein, we report the prevalence of MDROs in Libyan civil war victims on admission to the Northwest Medical Centre (NMC) in Frankfurt/Main, Germany, during a 6-month period from 2016 to 2017. Transfer and medical treatment took place based on an ongoing arrangement with the officially recognized Libyan government and under the auspices of the Federal German Foreign Ministry.

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Materials and Methods

This study was carried out at the NMC, Frankfurt/Main in cooperation with the Robert Koch-Institute (RKI), Werningerode, from August 2016 until January 2017. Upon admission, all patients were subjected to a preventive screening program for the presence of multidrug-resistant nonfermenters and Enterobacteriaceae, MRSA, and vancomycin-resistant enterococci (VRE). Culture of rectal, nasopharyngeal, and inguinal swabs was performed upon admission and repeated weekly in case of a prolonged inpatient stay. Similarly, wounds and other clinical sites of infection, if present, were investigated.

For MRSA detection, swabs were directly inoculated onto chromogenic MRSA-selective solid medium (BBL, CHROMagar MRSA II; Becton Dickinson, Heidelberg, Germany). For CRO and VRE screening, previously described methods[11–14] were used with some modifications. For CRO screening, swabs were directly plated on MacConkey medium (BBL; Becton Dickinson) followed by placing one 30 μg cefuroxime disk and two 10 μg carbapenem disks (ertapenem and meropenem; Becton Dickinson) on the inoculated plate. Similarly, for VRE screening, swabs were directly plated on Columbia colistin nalidixic acid (CNA) medium (BBL, Becton Dickinson), and one 30 μg vancomycin disk (Becton Dickinson) was then placed on the plates to select for vancomycin-dependent enterococci and VRE. Plates were read after 24 and 48 hours of incubation and controlled for growth and potential presence of suspicious colonies inside the expected inhibition zones according to Clinical and Laboratory Standards Institute (CLSI).[15]

Species identification and antimicrobial susceptibility testing were performed using standard laboratory protocols (*e.g.*, MicroScan WalkAway 96, Beckman Coulter, Krefeld, Germany; MALDI-TOF, Bruker, Bremen, Germany; disk diffusion, and antibiotic gradient tests, BioMérieux, Nürtlingen, Germany). Interpretation of results was carried out following the CLSI guidelines.[15]

All isolated Enterobacteriaceae, *Acinetobacter* spp., and *Pseudomonas* spp. were classified as 3MRGN or 4MRGN according to the phenotypic resistance definition issued by the German Commission for Hospital Hygiene and Infection Control (KRINKO). The term 3MRGN thereby refers to gram-negative bacteria resistant to three of four antibiotic groups (penicillins with piperacillin as a surrogate substance, cephalosporins with cefotaxime and/or ceftazidime as a surrogate substance, and fluoroquinolones with ciprofloxacin as a surrogate substance). Accordingly, 4MRGN refers to gram-negative bacteria with an additional or single resistance to carbapenems with imipenem, and/or meropenem as surrogate substances.[16]

Initial molecular testing of 4MRGN isolates for the most common carbapenemase genes was performed at NMC by multiplex PCR on a LightCycler 480 instrument (Roche, Mannheim, Germany) using commercially available reagents and specific primers (Roche and Tibmolbiol, Germany) to detect the most prevalent carbapenemase genes (bla_{KPC}, $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}48\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$, $bla_{IMP\text{-}like}$, $bla_{VIM\text{-}like}$, and $bla_{NDM\text{-}like}$) as previously described.[17,18]

To verify these results and to identify possible further mechanisms of resistance, additional evaluation of the same 4MRGN isolates for the presence of a second set of carbapenemase genes ($bla_{NDM\text{-}like}$, $bla_{OXA\text{-}48\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$, $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}51\text{-}like}$+*IS*AbaI, $bla_{VIM\text{-}like}$, $bla_{IMP\text{-}like}$, and $bla_{KPC\text{-}like}$) and for other β-lactamase genes ($bla_{TEM\text{-}like}$, $bla_{SHV\text{-}like}$, $bla_{CTX\text{-}M\text{-}Group\text{-}1\text{-}2\text{-}9}$, $bla_{OXA\text{-}1\text{-}like}$, $bla_{OXA\text{-}2\text{-}like}$, $bla_{OXA\text{-}9\text{-}like}$, $bla_{OXA\text{-}10\text{-}like}$, $bla_{CMY\text{-}like}$, and $bla_{DHA\text{-}like}$) was conducted at the National Reference Centre at RKI using PCR assays as described elsewhere.[18,19] Screening for plasmid-mediated quinolone resistance (PMQR) genes (*qnrA/B/S* and *aac(6′)Ib-cr*) and plasmid-mediated colistin resistance gene *mcr-1* was performed using previously published primers.[20,21]

All isolates were subjected to pulsed-field gel electrophoresis (PFGE) using *Xba*I-restricted (Enterobacteriaceae) and *Apa*I-restricted (*Acinetobacter baumannii*) whole genomic DNA. Macrorestriction patterns were interpreted according to the criteria described by Tenover *et al.*[22] *Escherichia coli* isolates were epidemiologically assigned to one of the four main phylogenetic groups (A, B1, B2, and D) and clonal lineage (O25b:H4-ST131) based on PCR assays.[23,24] All *A. baumannii* isolates were proven to belong to the frequent international clones (ICs) 1–3 (formerly European clones I–III) using a PCR-based assay.[25]

Results

Between August 2016 and January 2017, a total of 67 Libyan patients were admitted to NMC for in- or outpatient treatment and further medical evaluation. All but one were male with a median age of 29 years. The most common injuries were gunshot wounds and wounds from explosive devices resembling the typical injury pattern already known from previous reports on Libyan war victims[26] and requiring (neuro)surgical or neurological therapy. Sixty-four patients were admitted to hospitals in Libya and/or in Tunisia for primary treatment, whereas three patients (Table 2: Nos. 8, 43, and 52) were temporarily treated in Turkish hospitals.

During initial admission to NMC, MDROs were found in 40 (60%) patients, including 8 (12%) individuals who were tested positive for 3MRGN and 25 (37%) patients with at least one 4MRGN. MRSA was found in 11 (16%) patients, whereas none of the patients showed colonization with VRE. Five (7%) patients presented with two different types of MDROs (Table 1). Of the 40 patients colonized with MDROs, only 10 displayed clinical signs of infection with the respective microorganism. Most frequently involved infection sites were the urinary tract, the respiratory tract, bone, soft tissues, and wounds. Three of the 10 clinically infected patients had a positive intraoperative culture from deep (orthopedic) lesions.

A total of 39 of the 41 CRO isolates were tested for molecular mechanisms of resistance (one *A. baumannii* and one *Pseudomonas aeruginosa* were not available for further analysis). Fifteen isolates of *A. baumannii* demonstrated production of a carbapenemase: OXA-23 ($n=9$), NDM ($n=2$), and NDM + OXA-51-*IS*AbaI ($n=4$). Nineteen of 21 isolates of *Klebsiella pneumoniae* harbored carbapenemase genes $bla_{OXA\text{-}48}$ ($n=9$), bla_{NDM} ($n=6$), and $bla_{OXA\text{-}48}$ + bla_{NDM} ($n=4$). The two noncarbapenemase-positive CRO-*K. pneumoniae* isolates were extended-spectrum β-lactamase (ESBL) producers and demonstrated ertapenem resistance and reduced susceptibility to imipenem and meropenem. Subsequent

Table 1. Multidrug-Resistant Organisms Status upon Initial Admission of 67 Libyan War Casualties Transferred to Northwest Medical Centre, Frankfurt/Main, Germany

MDROs	*Patients,* n *(%)*
MDROs any	40 (60)
3MRGN only	8 (12)
4MRGN	25 (37)
≥2 different species of 4MRGN	13 (19)
MRSA	11 (16)
VRE	0
Presence of two types of MDROs: MRSA and 3MRGN or 4MRGN	5 (7)

MDROs include MRSA, VRE, and 3MRGN and 4MRGN bacteria. 3MRGN and 4MRGN bacteria; 3MRGN: multidrug-resistant gram-negative bacteria with resistance to penicillins (piperacillin), cephalosporins (ceftazidime and/or cefotaxime), and fluoroquinolones (ciprofloxacin); 4MRGN: multidrug-resistant gram-negative bacteria with resistance to penicillins (piperacillin), cephalosporins (ceftazidime and/or cefotaxime), fluoroquinolones (ciprofloxacin), and carbapenems (imipenem and/or meropenem); MDROs, multidrug-resistant organisms; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci.

sequencing of porin genes *ompK35* and *ompK36*[18] of these two CRO-*K. pneumoniae* revealed premature STOP codons in both genes leading to incomplete porins or complete loss of the porin. Furthermore, one OXA-48-producing *E. coli*, one NDM-producing *Enterobacter cloacae*, and one OXA-48-positive *Serratia marcescens* were detected (Table 2). There was 100% concordance between molecular test results of carbapenemase genes at the NMC and the RKI.

Most of the 4MRGN Enterobacteriaceae were additionally positive for ESBL of CTX-M-group-1 and other β-lactamases (TEM-type, SHV-type, OXA-1, and OXA-9). Furthermore, PMQR genes *qnrB* ($n=4$) and *aac(6′)-Ib-cr* ($n=24$) were detected in carbapenem-resistant *K. pneumoniae*, *A. baumannii*, and *E. coli* (Table 2). The 15 carbapenemase-producing *A. baumannii* could be assigned to eight PFGE types, whereas PFGE types 1–4 could be assigned to IC 2. Among 21 isolates of carbapenem-resistant *K. pneumoniae*, 5 different PFGE patterns were identified and the OXA-48-positive *E. coli* was assigned to phylogenetic group B2 (Table 2). The plasmid-mediated colistin resistance gene *mcr-1* was not identified in any of the 4MRGN isolates.

Discussion

There is good evidence for a linkage between combat-associated injuries and patient colonization or infection with MDROs, especially with MDR-*A. baumannii*, MDR-*P. aeruginosa*, and MDR-*K. pneumoniae*.[27–29] Proposed sources of these MDROs include pre-existing fecal or cutaneous colonization of the patient, inoculation into wounds at the time of injury from environmental contamination, and nosocomial transmission during treatment of patients within healthcare systems.[30] Whether the strains of our cohort represent environmental or hospital-acquired isolates cannot be definitively determined, as complete patient histories including information on all medical institutions involved were difficult to obtain. Our finding of some clonally related strains may be suggestive of nosocomial transmission but could also be indicative of influx of successful clones from the community. MDRO outbreaks at the NMC have been prevented by implementing strict infection prevention and control measures.[31]

In a recent study, 31 (69%) of 45 Syrian war-injured patients with clinically confirmed surgical site infection were positive for MDROs.[32] In particular, patients and soldiers who experienced delays in definite wound management were frequently found positive for MRSA and other MDROs such as ESBL-producing Enterobacteriaceae, *P. aeruginosa*, or *A. baumannii.* Similarly, a study from Libya demonstrated high incidence rates for MDR-*A. baumannii*, MDR-*P. aeruginosa*, and ESBL producers.[33] Our findings of MDROs in 60% of Libyan war victims transferred to Germany for medical aid are similar to reports from the Netherlands where MDROs (defined using the Dutch Working Group for Infection Prevention classification) were demonstrated in 25 (49%) of 51 Libyan war casualties admitted to the Major Incident Hospital in Utrecht.[3] Risk factors associated with the colonization by MDROs in the Dutch study were (1) previous hospital admission in Libya, (2) previous antibiotic treatment in Libya, and (3) the presence of open wounds.

A lower detection rate of MDROs (51 [24%] of 213 Libyan patients) was published in another study from Germany.[34] Since treatment for that cohort was mostly for nonacute diseases secondary to war injuries, one might speculate that those patients lost carriage of MDROs although it is known that prolonged carriage can occur in a substantial proportion of such patients.[35] As already published by the Dutch study, a single set of screening cultures upon admission does not suffice for a reliable characterization of the patients' MDRO status.[3] Single culture screening is not 100% sensitive and presents diagnostic limitations for accurate detection of OXA-48-producers.[36] Of concern, we experienced three patients exhibiting additional species of MDROs when hospital follow-up was compared with the initial screening.

The southern Mediterranean basin is recognized as an area of high prevalence for MRSA, most notably in patients with burns and surgical wound infections.[37] Interestingly, our investigation corroborates data of two other studies[3,34] and reveals relatively low colonization rates for MRSA (16%, 10%, and 8%, respectively). Similarly, we did not detect VRE. Nevertheless it is well established that in contrast to increasing trends in Europe and Germany, glycopeptide resistance in enterococci remains insignificant in Northern Africa.[38]

Carbapenem resistance can be conferred by the presence of various mechanisms, including carbapenemases. Thirty-seven of the 39 carbapenem-resistant isolates were positive for single or combined presence of carbapenemase genes in *E. coli*, *E. cloacae*, *A. baumannii*, *K. pneumoniae*, and *S. marcescens* (bla_{NDM}, bla_{OXA-48}, bla_{OXA-23}, and $bla_{OXA-51-ISAbaI}$) (Table 2). These findings expand on the existing literature stating that North Africa is mainly considered a reservoir of oxacillinase producers, particularly OXA-48 in Enterobacteriaceae[39] and OXA-23 in *A. baumannii.*[40] The first sporadic cases of metallo-β-lactamases such as NDM and class A carbapenemases such as KPC have only been reported quite recently in North Africa,[41,42] so these findings add evidence to a potentially changing and possibly worsening resistance epidemiology in the closely linked and neighboring countries of Tunisia and Libya.

MDROs can influence mortality, morbidity, duration of hospitalization, and cost of care with an adverse impact on

Table 2. Molecular Characteristics of Multidrug-Resistant Gram-Negative Bacteria ($N = 39$) from Libyan Patients

Patient no.	*Carbapenemase(s)*	*Other β-lactamases*	*PMQR genes*	*PCR clone*[a]	ApaI-*PFGE-type*
Acinetobacter baumannii *(n = 15)*					
4	OXA-23	—	—	n.t.	A-6a
5	OXA-23	—	—	EU-II/IC 2	A-4
16	OXA-23	—	—	EU-II/IC 2	A-1
18	OXA-23	—	—	n.t.	A-6
21	OXA-23	—	—	EU-II/IC 2	A-1
22	NDM, OXA-51-*IS*AbaI	TEM	—	n.t.	A-5
23	NDM	—	—	EU-II/IC 2	A-2
24	OXA-23	—	—	EU-II/IC 2	A-1
28	NDM, OXA-51-*IS*AbaI	TEM	—	n.t.	A-5
30	OXA-23	—	*aac(6′)Ib-cr*	EU-II/IC 2	A-3
53	OXA-23	—	—	EU-II/IC 2	A-1
56	NDM, OXA-51-*IS*AbaI	—	—	n.t.	A-5a
58	NDM	—	—	n.t.	A-7
59	OXA-23	TEM	*aac(6′)Ib-cr*	n.t.	A-8
62	NDM, OXA-51-*IS*AbaI	—	—	n.t.	A-5

	Carbapenemase(s)	*Other β-lactamases*	*PMQR genes*	*Phylogenetic group*	XbaI-*PFGE-type*
Escherichia coli *(n = 1)*					
8	OXA-48	SHV	—	B2	Eco-1

	Carbapenemase(s)	*Other β-lactamases*	*PMQR genes*	XbaI-*PFGE-type*
Enterobacter cloacae (n = 1)				
20	NDM	TEM, OXA-1, CTX-M-group-1	*aac(6′)Ib-cr*	Ecl-1
Serratia marcescens *(n = 1)*				
62	OXA-48	—	—	S-1

	Carbapenemase(s)	*Other β-lactamases*	*PMQR genes*	XbaI-*PFGE-type*
Klebsiella pneumoniae *(n = 21)*				
4	OXA-48, NDM	SHV, OXA-1, CTX-M-Group-1	*aac(6′)Ib-cr*	K-3a
8	OXA-48	TEM, SHV, OXA-1, OXA-9, CTX-M-group-1	*aac(6′)Ib-cr*	K-4b
15	OXA-48	TEM, SHV, OXA-1, OXA-9, CTX-M-group-1	*aac(6′)Ib-cr*	K-4 + K-4a[b]
18	OXA-48, NDM	TEM, SHV, OXA-1, CTX-M-group-1	*qnrB, aac(6′)Ib-cr*	K-2a
21	OXA-48	TEM, SHV, OXA-1, OXA-9, CTX-M-group-1	*aac(6′)Ib-cr*	K-4c
22	OXA-48	TEM, SHV, OXA-1, OXA-9, CTX-M-group-1	*aac(6′)Ib-cr*	K-4
24	OXA-48	TEM, SHV, OXA-1, OXA-9, CTX-M-group-1	*aac(6′)Ib-cr*	K-4
24	NDM	SHV, OXA-1, CTX-M-group-1	*aac(6′)Ib-cr*	K-3a
43	—	TEM, SHV, OXA-1, OXA-9, CTX-M-group-1	*aac(6′)Ib-cr*	K-4e + K4f[b]
44	OXA-48	TEM, SHV, OXA-1, CTX-M-group-1	*aac(6′)Ib-cr*	K-5
44	OXA-48	TEM, SHV, OXA-1, CTX-M-group-1	*aac(6′)Ib-cr*	K-5a
45	NDM	TEM, SHV, OXA-1, CTX-M-group-1	*qnrB, aac(6′)Ib-cr*	K-2
49	NDM	SHV, OXA-1, CTX-M-group-1	*aac(6′)Ib-cr*	K-1
52	NDM	SHV, OXA-1, CTX-M-group-1	*aac(6′)Ib-cr*	K-3
53	NDM	TEM, SHV, OXA-1, CTX-M-Group-1	*qnrB, aac(6′)Ib-cr*	K-2b
55	OXA-48, NDM	SHV, OXA-1, CTX-M-group-1	*aac(6′)Ib-cr*	K-3c
56	OXA-48	TEM, SHV, OXA-1, OXA-9, CTX-M-group-1	*aac(6′)Ib-cr*	K-4
58	NDM	TEM, SHV, OXA-1, OXA-9, CTX-M-group-1	*qnrB, aac(6′)Ib-cr*	K-2a
59	—	TEM, SHV, OXA-1, OXA-9, CTX-M-group-1	*aac(6′)Ib-cr*	K-4d
62	OXA-48	TEM, SHV, OXA-1, CTX-M-group1	*aac(6′)Ib-cr*	K-4c
65	OXA-48, NDM	SHV, OXA-1, CTX-M-group-1	*aac(6′)Ib-cr*	K-3b

[a]PCR clones as described by Turton *et al.*[22]
[b]Two different morphotypes were selected.
n.t., nontypeable; PFGE, pulsed-field gel electrophoresis; PMQR, plasmid-mediated quinolone resistance.

individuals and societies.[43] Although reports of MDROs after humanitarian medical treatment of Libyan combatants are relatively scarce, these reports clearly point to increasing risks resulting from a continuous spread of MDROs into Europe from cross-border transfer of such patients. The first documented case of an OXA-48-producing *K. pneumoniae* in Slovenia was recently reported in a patient transferred from Libya for further medical assistance.[44] Similarly, among 45 patients transferred from Libya to Denmark, 7 carried OXA-48-producing *K. pneumoniae*, 1 carried an NDM-1-producing *A. baumannii*, 5 carried MRSA, and 3 patients had OXA-23-producing *A. baumannii* in their screening cultures upon hospital admission. This was the first description of OXA-48-producing *K. pneumoniae* and NDM-1-producing *A. baumannii* in Denmark.[2] The steady influx of injured refugees from Libya may have a significant impact on the increase in OXA-48-producing Enterobacteriaceae in Malta.[45] In parallel, several other case reports from Italy, Switzerland, France, and the United Kingdom describe an influx of carbapenemase-producing Enterobacteriaceae.[46–49] Therefore, patients from Libya and the surrounding region must be seen as both possible victims and vectors of MDROs.[50]

It is well known that a link exists between spread of antibiotic resistance and many of the so-called intercountry patients.[51,52] Consequently, a 2011 technical report published by the European Centre for Disease Prevention and Control stated that providing healthcare to patients transferred from countries where MDROs are endemic is associated with a substantial risk of MDRO transmission and spread.[53] Our finding of high MDRO rates in Libyan war casualties should raise awareness and serve as a warning for strict compliance of infection control protocols in medical institutions that are involved in the care of these patients.

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