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Epidemiology and antimicrobial susceptibilities of wound isolates of obligate anaerobes from combat casualties[☆]

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ABSTRACT

Data from recent conflicts related to war wounds and obligate anaerobes are limited. We define the epidemiology and antimicrobial susceptibility of obligate anaerobes from Iraq and Afghanistan casualties (6/2009–12/2013), as well as their association with clinical outcomes. Susceptibility against eleven antibiotics (7 classes) was tested. Overall, 59 patients had 119 obligate anaerobes identified (83 were first isolates). Obligate anaerobes were isolated 7–13 days post-injury, primarily from lower extremity wounds (43%), and were largely *Bacteroides* spp. (42%) and *Clostridium* spp. (19%). Patients with pelvic wounds were more likely to have *Bacteroides* spp. and concomitant resistant gram-negative aerobes. Seventy-three percent of isolates were resistant to ≥1 antimicrobials. *Bacteroides* spp. demonstrated the most resistance (16% of first isolates). Patients with resistant isolates had similar outcomes to those with susceptible strains. Serial recovery of isolates occurred in 15% of patients and was significantly associated with isolation of *Bacteroides* spp., along with resistant gram-negative aerobes.

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1. Introduction

Military personnel wounded in Iraq and Afghanistan and admitted to an intensive care unit (ICU) during initial hospitalization had infection rates as high as 50% (Tribble et al., 2011). These infections have been commonly caused by multidrug-resistant (MDR) gram-negative aerobes, methicillin-resistant *Staphylococcus aureus*, and invasive molds; however, obligate anaerobes have also been reported (1.2–1.4%) (Murray et al., 2009a, 2011; Tribble et al., 2011; Warkentien et al., 2012).

During World War I, *Clostridium* spp. was found in >80% of wounds within the first 9 days post-injury (Fleming, 1915). A study of war wound flora revealed that *Clostridium* spp. was isolated from 52% of wounds within 3 days of injury, 18% between days 4–12, and no isolation after 12 days (Miles et al., 1940). In wounds from the Korean War, *Clostridium* spp. was recovered within 4.5 hours after injury from 36–84% (Lindberg et al., 1955). A study of 63 wounds from the Vietnam War revealed 3% were associated with anaerobes (*Clostridium* spp. and *Bacteroides* spp.) identified on the day of injury (Tong, 1972). Overall, gas gangrene rates (likely caused by *Clostridium* spp.) decreased from 5% (28% mortality) in World War I to 0.3–1.5% (15% mortality) in World War II to 0.08% (no mortality) in the Korean War and to no cases in the Vietnam War (Murray et al., 2008). This decreasing incidence and mortality is likely reflective of improved evacuation times, surgical techniques, and antimicrobial therapy. Nonetheless, the clinical implication of isolation of any obligate anaerobes from wounds has been questioned, recognizing that the presence of anaerobes does not always indicate infection (Lindberg et al., 1955; MacLennan, 1943).

Although there has been at least one case report of a casualty with a resistant isolate, obligate anaerobic bacteria are rarely evaluated for antimicrobial resistance, limiting the ability to empirically select the ideal antimicrobial coverage, (Murray et al., 2009b; Sherwood et al., 2011). Furthermore, it is not clear if antimicrobial resistance of obligate anaerobes is associated with poor outcomes (Bowler et al., 2001). This study was designed to identify the epidemiology, resistance patterns, and the impact of obligate anaerobes isolated from combat casualties.

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2. Materials and methods

2.1. Study population and demographics

Utilizing data obtained through the Trauma Infectious Disease Outcomes Study (TIDOS) (Tribble et al., 2011), wounded military personnel medically evacuated from Iraq and Afghanistan combat theaters to Germany (1 June 2009–31 December 2013) with recovered obligate anaerobes archived in the TIDOS microbiological repository were evaluated. Sites of pathogen isolation were categorized as upper extremity, lower extremity, trunk/head, pelvic, and other. Wounds of mid-distal thigh were included in lower extremity wounds, while those of proximal thigh were included with pelvic. Trunk and head wounds were defined as superficial wounds of the abdominal wall (extra-peritoneal), chest wall, and back, along with soft-tissue injury of the head. Given the low numbers of intra-abdominal, central nervous system, respiratory, and bloodstream infections, these were not analyzed individually. The database was queried to see if these patients had co-isolation with MDR aerobes (e.g., methicillin-resistant *Staphylococcus aureus*, *Pseudomonas, Acinetobacter,* vancomycin-resistant *Enterococcus*, and extended spectrum beta-lactamase-producing Enterobacteriaceae) or invasive molds as well as for serial bacterial isolation as a marker for absence of wound clearance and mortality.

2.2. Clinical microbiology and antimicrobial susceptibility testing

Isolates were recovered following clinical workups per standard practices at the military hospital clinical laboratories and archived in the TIDOS microbiological repository. Archived isolates were grown under anaerobic conditions [using Advanced Anoxomat system (Advanced Instruments Inc., Norwood, MA)], on commercially available OxyPRAS Plus Brucella Blood Agar (VWR, Sugar Land, TX), using the agar dilution method as described by the Clinical and Laboratory Standards Institute (CLSI) and a Steers replicator (CLSI, 2012). Isolates with morphologies not matching the organism were confirmed via gram stain, aerotolerance test, and Remel RapID ANA II System (Remel Inc., Lenexa, KS) to verify speciation. Antimicrobials tested represented seven different classes with established breakpoints for obligate anaerobes: beta-lactam/beta-lactamase inhibitor, cephalosporin, carbapenem, metronidazole, clindamycin, moxifloxacin, and tigecycline in addition to linezolid (no established breakpoint). Testing was performed on fresh (<24 hours) Brucella agar supplemented with hemin (5 mcg/ml), vitamin K (1 mcg/ml) and 5% (v/v) laked sheep blood and containing various concentrations of antimicrobials: ampicillin-sulbactam (SIGMA-Aldrich, St. Louis, MO) at 4/2, 8/4, 16/8, 32/16, and 64/32 mcg/ml; cefoxitin (SIGMA-Aldrich, St. Louis, MO) at 8, 16, 32, 64, and 128 mcg/ml; clindamycin (MP Biomedicals LLC, Solon, OH) at 1, 2, 4, 8, and 16 mcg/ml; ertapenem (Merck & Co., Inc., Whitehouse Station, NJ) at 2, 4, 8, 16, and 32 mcg/ml; imipenem (SIGMA-Aldrich, St. Louis, MO) at 2, 4, 8, 16, and 32 mcg/ml; linezolid (SIGMA-Aldrich, St. Louis, MO) at 0.5, 1, 2, 4, 8, 16, and 32 mcg/ml; meropenem (SIGMA-Aldrich, St. Louis, MO) at 2, 4, 8, 16, and 32 mcg/ml; metronidazole (SIGMA-Aldrich, St. Louis, MO) at 4, 8, 16, 32, and 64 mcg/ml; moxifloxacin (SIGMA-Aldrich, St. Louis, MO) at 1, 2, 4, 8, and 16 mcg/ml; piperacillin-tazobactam (SIGMA-Aldrich, St. Louis, MO) at 16/4, 32/4, 64/4, 128/4, and 256/4 mcg/ml; and tigecycline (SIGMA-Aldrich, St. Louis, MO) at 2, 4, 8, 16, and 32 mcg/ml. Quality control strains (*Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, *Eggerthella lenta* ATCC 43055, *Clostridium difficile* ATCC 700057) grew as expected. Due to swarming *Clostridium* spp., segmented agar plates were implemented (VWR, Sugar Land, TX). Minimum inhibitory concentrations (MICs) were determined after incubation for 48 hours and applied to breakpoints from CLSI except for linezolid (CLSI, 2012), or package insert data (tigecycline) to obtain susceptibilities. MIC_{50} and MIC_{90} were defined as MICs necessary to inhibit growth among 50% or 90% of that group of bacteria, respectively. Resistance was compared with prior therapy and clinical outcomes, including serial recovery of the same organism from the same site and death. This study was approved by the Uniformed Services University Infectious Disease Institutional Review Board.

2.3. Statistical analysis and definitions

Chi-square analysis was performed on categorical variables and Mann-Whitney *U* tests were utilized for continuous variables to compare wound sites with clinical and demographic information. Statistical significance was defined as a P value of <0.05. The term 'initial isolate' refers to the first time that a particular species was recovered from a source, and was collected at time of clinical concern for active infection. Site is defined as location of the source of the isolate (each wound represented as a different site). Serial recovery is defined as isolating the same species from the same site after a period of ≥48 hours after the initial isolate. Organisms were classified as MDR if they were resistant to ≥3 antibiotic classes.

3. Results

3.1. Study population and demographics

A total of 4180 US combat casualties were medically evacuated to Germany during the study period, of which 59 (1.4%) had growth of obligate anaerobes on culture. The patients were severely injured, as indicated by high injury severity scores (ISS; median 21), ICU requirement (97%), and prolonged hospitalization (median 61 days; Table 1). No particular wound site was significantly associated with ICU admission, death, concomitant MDR aerobic bacteria recovery, or mold infection. Patients with pelvic wounds had a significantly higher ISS (median 30, $P < 0.01$) and longer hospitalization (median 122 days, $P < 0.01$).

There were 5799 isolates collected from wounded military personnel and archived in the TIDOS microbiological repository. Among these isolates, 123 (2.1%) were obligate anaerobes (69 patients, 1.7%); however, 19 were excluded due to lack of growth, resulting in 104 (1.8%) isolates from 59 patients available for evaluation. Eighty-three initial isolates were analyzed (Table 2). Seventy (84%) were from wound specimens, primarily of the lower extremities (35; [42%]). Abdominal and trunk wounds were not associated with penetration of peritoneal or pleural cavities.

There were three documented intra-abdominal infections with obligate anaerobes (*Bacteroides fragilis*, *Bacteroides caccae*, *Clostridium innocuum*) from penetrating abdominal trauma (one non-improvised explosive device [IED] blast and two gunshot wounds). There were 7 isolates from blood (4 *Propionibacterium acnes*, 1 *Bacteroides fragilis*, 1 *Finegoldia magna*, 1 *Prevotella melaninogenica*), one *Propionibacterium acnes* isolate from cerebrospinal fluid, and one *Prevotella carpais* isolate from a respiratory culture. None of these patients died or had recurrence of recovered pathogen from those sites.

3.2. Clinical microbiology and antimicrobial susceptibility testing

Obligate anaerobes were initially isolated a median of 7–13 days post-injury, depending on the source (Table 2). Initial isolates consisted of 35 (42%) *Bacteroides* spp., 16 (19%) *Clostridium* spp., 11 (13%) *Prevotella* spp., 10 (12%) *Finegoldia magna*, 9 (11%) *Propionibacterium acnes*, and 2 (2%) *Peptostreptococcus* spp. Patients with *Bacteroides* spp. had the longest hospitalizations (median: 67, min–max: 17–257 days), and *Clostridium* spp. had the shortest time from injury to culture (median: 7.5, min–max: 3–11 days). All upper extremity wounds had *Clostridium* spp. *Bacteroides* spp. (11 of first isolates) frequently grew from pelvic wounds, but were not recovered from upper extremity wounds.

Overall, approximately 15% of the 4180 combat casualties developed a wound infection. Among the 59 patients with anaerobes, wound infections were identified in 50 (85%), of which 26 (52%) also had MDR aerobic bacteria collected (Table 1). Eight patients (14%) had concomitant

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