CASE SERIES

Case series of *Anaerococcus prevotii* infection in road traffic accidents in New Delhi, India

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ABSTRACT

Anaerobic bacteria are part of the normal human skin flora. However, anaerobic infections could have different clinical courses with outcomes ranging from local abscesses to life-threatening conditions. Any long-term wound infection or a wound discharging pus with a foul smell is an indication of anaerobic infections. We present here a series of three cases of infection in patients injured in road traffic accidents (RTA) in which *Anaerococcus prevotii* was identified as the pathogen.

Samples isolated from tissues of patients from three different RTAs were received in anaerobic conditions in Robertson Cooked Meat medium (RCM) within 2-4 h of collection. RCM was incubated for 24 h and then inoculated on three blood agars (BA) plates cultured anaerobically in a Gas Pack Jar, in a CO₂ incubator, or aerobically. No growth was noted on the aerobically incubated plate. After 48 h, anaerobic plates were examined for growth and a Gram stain was performed. The identity of isolated colonies was confirmed by VITEK-2, and sensitivity testing was done by the pour plate method using Epsilon meter strips. All three tissue samples isolated from (1) the right leg, (2) the right inguinal region, and (3) the left thigh of patients revealed *Anaerococcus prevotii*, and two isolates showed resistance to the antibiotic metronidazole.

Any injury with long-term infection, especially in patients suffered from RTAs, needs to be scrutinized for anaerobic infections since they are common in RTA related injuries. No random medication should be administered without prior culture sensitivity testing, because it might contribute to metronidazole or other antibiotics resistance.

Keywords: *Anaerococcus prevotii*, anaerobic infection, road traffic accident

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INTRODUCTION

Road traffic accidents (RTAs) are common in India, accounting for 218,876 deaths in 2017 with a standardized death rate of 17.2 (15.7 to 18.1) per 100,000 population [1]. The RTA injuries increase the probability of anaerobic infections.

Anaerobic soft tissue infections associated with RTA-related injuries are known for inflicting devastating tissue destruction and death. More than 80% of infected and 70% of non-infected leg ulcers harbor anaerobic organisms [2]. Anaerobic infections are characterized by tissue necrosis and suppuration. Some anaerobic bacteria, in synergy with aerobic or facultative bacteria, can resist oxygenated microenvironments and are known to proliferate in damaged tissue [3].
Clostridium spp. infection (gas gangrene) occurs commonly in muscle injury after contamination with soil or other foreign material containing the spores of C. perfringens, C. septicum, and some other bacterial species [4]. A study conducted in Hyderabad focused on Clostridium species infections in patients who experienced RTAs [5].

Many cases of infection caused by Anaerococcus prevotii affecting different body areas have been reported. These include septic arthritis in the native knee joint [6], and pneumonia with subsequent hematological spread causing osteomyelitis of the femur [7]. There have been various case reports of Anaerococcus prevotii infections but to the best of our knowledge, this bacterium was not identified as a causative agent of infection in patients with RTA-related injuries until now.

In order to make the right diagnosis in every case of infection from the RTA injury the infection agent requires proper identification and the consequent culture sensitivity testing for antibiotics is necessary. Any suspected anaerobic infection should be taken seriously, as these infections can quickly become fatal if left untreated. Here we are presenting a case series of three patients injured in RTA and developed soft tissue infection. We were able to isolate the infectious pathogen from these patients and identify it as Anaerococcus prevotii.

METHODS

All patients or their relatives who participated in this study have signed an informed consent. All tissue samples for anaerobic culture and sensitivity testing were sent in anaerobic conditions in Robertson’s Cooked Meat (RCM) medium to the microbiology laboratory (Fig. 1).

Fig. 1. Container with sample in RCM.

Tissue samples were minced with sterile scissors, submerged in a sufficient volume of pre-reduced broth to emulsify the specimen, and treated as a liquid specimen [8]. After a 24 h incubation of RCM at 37°C, Gram staining was performed (Fig. 2).

Fig. 2. Gram stain of Anaerococcus prevotii.

Then, bacterial samples were subcultured on blood agar plates at 37°C in a triplicate manner: one plate was incubated in aerobic conditions for 48 h, another plate – in the atmosphere containing 5-10% CO₂ in a CO₂ incubator for 48 h, and the third plate – in anaerobic conditions (sealed with parafilm) in a Gas Pack Jar for 72 h. The anaerobic plates showed colonies of grey color, nonhemolytic in nature. All of them were gram-positive cocci. The aerotolerance test (growing in the presence of oxygen) was negative. The plates incubated in aerobic conditions and in 5-10% CO₂ atmosphere showed no bacterial growth in 48 h. The colonies isolated on the anaerobic plate were analyzed using VITEK 2 system (Bio Merieux, France) and were confirmed to be Anaerococcus prevotii with a high probability. After confirmation of the isolate’s identity, antibiotic sensitivity tests were performed using the pour plate method with Epsilonmeter strips (Fig. 3) [9]. The antibiotic sensitivity tests and their interpretations were done according to CLSI, 32nd edition [10].

RESULTS

Case 1

A 40-year-old woman suffered a car accident leading to a lacerated wound injury of her left leg (Fig. 4). She was rushed to a hospital where wound debridement was done, and then received broad-spectrum antibiotics for 7 days with no response. On the 10th day, a tissue sample from the wound was sent for anaerobic culture and
antibiotic sensitivity testing. The complete blood count showed WBCs increasing continuously for 5 days, exceeding 20,000 cells/µl.

Case 3
A 35-year-old man was hit by a car while crossing the road and injured his left thigh. Wound debridement was done, and the patient received antibiotic treatment. However, his condition deteriorated, with the WBC count reaching 22,000 cells/µl, and he went into septic shock. The patient’s sample for anaerobic culture was received 15 days after. None of the patients received metronidazole. The results of the antibiotic sensitivity testing are presented in Table 1.

DISCUSSION
Anaerobic bacteria are part of the normal flora of human skin and mucosal membranes. Anaerobic infection is usually developing at the site of bacterial colonization and range from simple local abscesses to life-threatening infections and can become fatal. Clostridium bacteria species are commonly found in the post-traumatic wounds of patients [11]. Clostridium spp. belong to anaerobic or occasionally aerotolerant gram-positive rods, which can be found in soil, feces, sewage, and marine sediments [12].

There are many reported cases of body tissue infections (abscesses), blood infections, as well as pleural, and vaginal infections caused by Anaerococcus prevotii [13]. Dowd et al. isolated Anaerococcus spp. from the chronic diabetic foot ulcers which was confirmed by pyrosequencing [14]. La Scola et al. detected Anaerococcus spp. in blood cultures that was confirmed by mass spectrometry and 16S rRNA gene sequencing [15]. In another case report, Anaerococcus prevotii was described as a pathogen causing brain abscesses [16]. In a study by Daunaraite, culturing of the surgical wound sample from the patient’s thigh produced bacterial colonies identified as Anaerococcus prevotii [7]. Anaerococcus prevotii was also isolated from the knee aspirate of a patient, as reported in a study from the United Kingdom [6]. Anaerococcus prevotii has been identified as the causative agent responsible for different human infections in various other cases, but to the best of our knowledge, no case reports of Anaerococcus prevotii in patients suffered from RTA have been published as of now.

Investigating antibiotic resistance patterns, we made a noteworthy observation regarding Anaerococcus prevotii: resistance to metronidazole was observed in bacteria isolated from two out of three patients, one of which died. As reported by Hecht, antibiotics have been prescribed for a longer period of up to 20 days in the case of Anaerococcus prevotii infections [17]. However, the antimicrobial susceptibility testing of anaerobes is rarely performed in most laboratories due to the long incubation period and special conditions required.
Metronidazole’s mechanism of action is not fully understood to date. It is believed that the drug molecule diffuses into the microorganism, then it is reduced to form reactive radical intermediates (that is accomplished mostly by anaerobic bacteria) that interact with DNA and electron-transport proteins blocking the DNA synthesis and repair. Therefore, metronidazole leads to cell death in susceptible microorganisms. In our study, bacteria from two out of three cases were found to be resistant to the metronidazole. Poulet et al. also reported metronidazole resistance in bacteria that caused >30% of gram-positive anaerobic infections [18].

Although antimicrobial resistance profiles in bacteria are known to vary by geographical location, hospital centers, national antibiotic consumption, bacterial species, and type of specimens [19-21], it is still necessary to be aware of increasing antibiotic resistance in bacteria causing anaerobic infections.

**CONCLUSION**

There have been reports of *Anaerococcus prevotii* infections in different clinical cases other than injuries from RTAs. Here, we isolated *Anaerococcus prevotii* in three cases of patients with injuries from RTAs, with bacterial resistance to antibiotic metronidazole revealed in two cases. Although *Clostridium* species are common in RTA related injuries, *Anaerococcus* species can also play an important role as a pathogen in these cases and even lead to fatal outcomes, as seen in one of the cases described here. Alongside other studies, our data suggest that different anaerobic infections have started showing resistance to metronidazole. In order to prevent metronidazole resistance among anaerobes, the random use of nitroimidazole antibiotics should be strongly discouraged and an antibiotic stewardship program for anaerobic organisms should be initiated in every big hospital.

### Table 1. Antibiotic sensitivity patterns of *Anaerococcus prevotii* isolated from three cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Anaerobic culture</th>
<th>Antibiotic sensitivity pattern</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>CASE 1</td>
<td><em>Anaerococcus prevotii</em> (tissue culture)</td>
<td>S – Amoxyclav (4/2 µg/ml) Metronidazole (6 µg/ml) Ceftriaxone (1 µg/ml) Moxifloxacin (2 µg/ml) Clindamycin (2 µg/ml) R – Ampicillin (2 µg/ml) Tetracycline (4 µg/ml) Penicillin G (0.5 µg/ml) Imipenem (4 µg/ml)</td>
<td>The patient recovered after treatment with Amoxyclav and Ceftriaxone.</td>
</tr>
<tr>
<td>CASE 2</td>
<td><em>Anaerococcus prevotii</em> (tissue culture)</td>
<td>S – Amoxyclav (4/2 µg/ml) Moxifloxacin (2 µg/ml) Tetracycline (4 µg/ml) R – Metronidazole (&gt;256 µg/ml) Penicillin G (20 µg/ml) Ceftriaxone (64 µg/ml) Ampicillin (2 µg/ml) Imipenem (16 µg/ml) Clindamycin (8 µg/ml)</td>
<td>The patient recovered after treatment with Amoxyclav and Moxifloxacin.</td>
</tr>
<tr>
<td>CASE 3</td>
<td><em>Anaerococcus prevotii</em> (tissue culture)</td>
<td>S – Moxifloxacin (2 µg/ml) R – Metronidazole (32 µg/ml) Amoxyclav (16/8 µg/ml) Penicillin G (20 µg/ml) Ceftriaxone (64 µg/ml) Ampicillin (2 µg/ml) Imipenem (16 µg/ml) Clindamycin (8 µg/ml) Tetracycline (4 µg/ml),</td>
<td>Even after treatment with active drugs, the patient expired, probably due to the delay in the anaerobic culture results, and, consequently, late prescription of the active drugs.</td>
</tr>
</tbody>
</table>

*S – sensitive, R – resistant*
REFERENCES


10. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 32nd ed.


